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Direct-Fed Microbials and Prebiotics for Animals

Science and Mechanisms of Action

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This book is dedicated to the late Dr. Stanley Gilliland, Regents Professor and Sitlington Endowed Chair in Food Microbiology at Oklahoma State University. Dr. Gilliland had an incredible career and was a pioneer in the fields of probiotics and direct-fed microbials. He mentored many graduate students and paved the way for the further advancement of the field of direct-fed microbials through his teaching and research. Dr. Gilliland contributed to many journal articles, book chapters, and conferences during his career. He left behind a legacy of research and the inspiration for quality scientific work. Dr. Gilliland will be missed but his contributions will not be forgotten.

Without Stan's guidance and support this conference and book would not have taken place. While Dr. Gilliland was taken from us too soon, his impacts will be felt for years to come.

Preface

In recent years, the role of the microbial ecosystem in both human and animal health has become more prominent (Finegold 2008; Ley et al. 2006; Murphy 2004; Turnbaugh et al. 2009; Turnbaugh et al. 2006; Xu and Gordon 2003). The "microbial organ" is at last getting its due as a playing a part in health as well as production parameters (Lyte 2010). Though much of this research has focused on the effects of the microbial communities and cross-communication with the host in and on humans, increasing amounts of research has delved into the microbial organ of animals (Freestone and Lyte 2010). A new hypothesis has recently been advanced by Dr. Mark Lyte that probiotics may function as a drug as a delivery mechanism for neuroactive and bioactive compounds that affect the host.

In light of these changes in our understanding of intestinal microbial ecology based on new molecular and older culture-based methods, a revised vision of the role of Direct fed Microbials and prebiotics in animal agriculture was necessary. With this in mind, an American Dairy Science Association DISCOVER conference was held in 2009 on "Probiotics in Animal Agriculture: Science and Mechanisms of Action". Following discussions with Dr. Gilliland and others at that conference, it was decided that a "state of the art" book needed to be produced for the animal and DFM industries.

The practice of supplementing direct fed microbial and prebiotic additives to domestic animals during growth is becoming more widespread in food animal production. Beneficial effects particularly in cattle, pigs and poultry including improved general health, foodborne pathogen reduction, more efficient food utilization, faster growth rate and increased milk and egg production continue to be reported. The success associated with direct fed microbial and prebiotic applications in multiple species ensures their continued commercialization and widespread use of such additives. However, several fundamental questions remain. It appears that early establishment and retention of an ecological balance in the gastrointestinal tract is an important first step for an external biological additive to be effective in young animals. Therefore, it is possible that the effectiveness of direct fed microbials and prebiotics in some animal species may only be an indirect consequence of speeding up the establishment of the dominant microflora characteristic of the adult

gastrointestinal tract. Consequently an understanding of the key processes during establishment of microflora in the gastrointestinal system that lead to the subsequent fermentation characteristics and ecological balance exhibited by the highly protective microflora is needed. Identifying these processes should lead to continued improvement in the effectiveness of available commercial products. Several additional areas of future research directions are also likely needed for further development and implementation of these biologicals.

A critical area that is now becoming possible is the rapid identification *in vivo* of characteristic microbial profiles to confirm successful establishment. Such techniques involve incorporation of molecular fingerprinting of both externally introduced cultures as well as the indigenous gastrointestinal microflora. This may also potentially help to achieve a better understanding of the mechanism(s) required for successful selection and optimization of direct fed microbials and prebiotics. In addition, this will provide insight into environmental factors that may play a role in the ability of direct fed microbials to limit pathogen transmission. Other arenas in which direct fed microbials and prebiotics may be important are in limiting establishment of pathogens in older animals which possess a more mature and developed gut microflora and need removal of pathogens already colonized in animal gastrointestinal tracts. Here success will be dependent on a much more complete picture of gastrointestinal microbial ecology and may include organisms which have been overlooked when typical direct fed microflora have been identified and characterized. In addition, modeling of microbial interactions in the gastrointestinal tract may be important to identify common factors within the complex matrix of the microbial consortium which help to serve as a barrier to prevent pathogens from coexisting with these microorganisms. Continued research on direct fed microbials and prebiotics in general should markedly expand their commercial applications.

Definitions

In this book, we use an overarching definition for **probiotics** as "a preparation or a product containing viable, defined microorganisms in sufficient numbers, which alter the micro-flora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host"(Schrezenmeir and De Vrese 2001). **Direct-fed microbials** (DFM) are a category of probiotics that are used in the animal industry in the United States (Fuller 1989; Schrezenmeir and De Vrese 2001). Typically, DFM as a category includes: traditional "probiotics" (live bacterial, fungal or yeast cultures), non-viable bacterial, fungal or yeast cultures, or endproducts of bacterial, fungal or yeast fermentations. Some of these products include cultures that utilize a mechanism of action similar to **Competitive Exclusion Cultures**, but are not included in that FDA definition (CVM 1997). **Prebiotics** are defined as non-living compounds that can be degraded by the intestinal microflora, and are often considered a "colonic food"(Collins and Gibson 1999; Crittenden 1999; Schrezenmeir and De Vrese 2001). Many of the yeast products (DFM) used

in the animal industry contain endproducts of fermentation that are prebiotics, or prebiotic like, which can explain some of the effects of those products on the microbial population of the intestinal tract.

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References

Collins, D.M., and G.R. Gibson . 1999. Probiotics, prebiotics, and synbiotics: Approaches for modulating the microbial ecology of the gut. *American Journal of Clinical Nutrition* 69:1052S-1057S.

Crittenden, R.G. 1999. Prebiotics. In *Probiotics: A critical review,* ed. G.W. Tannock, 141–156. Horizon Scientific Press: Wymondham.

CVM. 1997. VM Policy on Competitive Exclusion Products. (FDA, Center for Veterinary Medicine, Office of Management and Communications, Rockville.

Finegold, S.M. 2008. Therapy and epidemiology of autism-clostridial spores as key elements. *Medical Hypothesis* 70:508–511.

Freestone P., and M. Lyte. 2010. Stress and microbial endocrinology: Prospects for ruminant nutrition. *Animal* 4:1248–1257.

Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology* 66:365–378.

Ley, R.E., P.J. Turnbaugh, S. Klein, and J.I. Gordon. 2006. Human gut microbes associated with obesity. *Nature* 444:1022–1023.

Lyte, M. 2010. The microbial organ in the gut as a driver of homeostasis and disease. *Medical Hypothesis* 74:634–638.

Murphy, M. 2004. Bacteria could treat symptoms of autism. *Chemical Industries* (London):6.

Schrezenmeir, J., and M. De Vrese. 2001. Probiotics, prebiotics, and synbiotics-approaching a definition. *American Journal of Clinical Nutrition* 73(Suppl.):354s-361s.

Turnbaugh, P.J., M. Hamady, T. Yatsunenko et al. 2009. A core gut microbiome in obese and lean twins. *Nature* 457:480–484.

Turnbaugh, P.J., R.E. Ley, M.A. Mahowald, V. Magrini, E.R. Mardis, and J.I. Gordon. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444:1027–131.

Xu, J., and J.I. Gordon. 2003. Honor thy symbionts. *Proceedings of National Academy of Sciences of the United States of America* 100:10452–10459.

Contents

About the Editors

Dr. Todd R. Callaway received his B.S. Agriculture degree in Animal Health and Animal and Dairy Sciences, as well as his M.S. in Animal and Dairy Sciences from the University of Georgia. He received his Ph.D. in Microbiology from Cornell University, with minors in Biochemistry and Animal Science. Dr. Callaway is a Research Microbiologist for the Agricultural Research Service of the U.S. Department of Agriculture, and has served as a Science Fellow for the Foreign Agriculture Service and the U.S. State Department. He has adjunct faculty appointments in the Department of Agriculture at Angelo State University, the Animal and Dairy Sciences Department at Mississippi State University, and the Department of Animal Science at Texas A&M University. Dr. Callaway has a research program focused on manipulating the microbial ecology of the animal gastrointestinal tract to reduce populations of foodborne pathogenic bacteria in food animals before they enter the food chain.

Dr. Steven C. Ricke received his B.S. degree in Animal Science and M.S. degree in Ruminant Nutrition from the University of Illinois and his Ph.D. degree from the University of Wisconsin with a co-major in Animal Science and Bacteriology. He is currently holder of the Donald "Buddy" Wray Endowed Chair in Food Safety and Director of the Center for Food Safety at the University of Arkansas. He is also a faculty member of the Department of Food Science, the Department of Poultry Science and the Cellular and Molecular Graduate program. Dr. Ricke's research program is primarily focused on virulence and pathogenic characteristics of food-borne salmonellae.

Part I
Overview of Direct-Fed Microbials and Prebiotics and their Interactions with the Host

Chapter 1
The Commensal Microbiota

John A. Patterson

Abstract The commensal microbiota in the intestinal tract are important to the host, not only in relation to food digestion, but also in terms of reducing infection by pathogens (colonization resistance) and it is becoming increasingly apparent that the commensal microbiota are important in developmental programming and function of organ systems in the adult. This is not surprising as there are ten times as many microbial cells as host cells and 100 times as many microbial genes. The commensal microbiota could be considered an additional organ that not only influences function in the adult, but also development in the neonate. The commensal microbiota is an important component of the host animal's genome. During and immediately after birth, the intestinal tract is colonized by a succession of bacteria. The presence of these bacteria is important for functional development of the intestinal tract (angiogenesis, epithelial tissues, mucosal system) and more recent data suggests a role in development and function of the brain and hypothalamic pituitary axis (HPA) that last throughout life.

The commensal microbiota in the intestinal tract are important to the host, not only in relation to food digestion but also in terms of reducing infection by pathogens (colonization resistance). It is also becoming increasingly apparent that the commensal microbiota are important in developmental programming and function of organ systems in the adult. This is not surprising as there are ten times as many microbial cells as host cells and 100 times as many microbial genes (Gill et al. 2006). The commensal microbiota could be considered an additional organ that influences not only function in the adult but also development in the neonate (O'Hara and Shanahan 2006). The commensal microbiota comprise an important component of the host animal's genome.

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During and immediately after birth, the intestinal tract is colonized by a succession of bacteria. The presence of these bacteria is important for functional development of the intestinal tract (angiogenesis, epithelial tissues, mucosal system), and more recent data suggest a role in the development and function of the brain and the hypothalamic–pituitary axis (HPA) that lasts throughout life. From an ecological perspective, the intestinal tract could also be viewed as a major river running through a continent, originating in the headwaters and discharging after passing through the continent. The river ecosystem is dynamic and constantly changing and is influenced by the surrounding land, as the land is affected by the river. The intestinal tract has major (e.g., rumen in ruminants; crop, proventriculus in birds) and minor (human, mouse) differences in stomach structure that influence subsequent microbial ecosystems. There are major differences between species in the structure of the small intestine, cecum, and large intestine that also influence the dynamics of the microbial ecosystem. Individual differences in intestinal structure, pH, transit rate, water content, immune function, and expression of molecules lining the epithelium influence the unique microbiota in individual animals.

From a microbial ecological perspective, disturbances of ecosystems decrease microbial diversity and increase opportunities for invading species. For example, pasture soil may contain 3,500–8,800 bacterial species, whereas species diversity in arable land comprises some 140–350 species (Horner-Devine et al. 2004; Xavier et al. 2005). The luminal contents of the intestinal tract are constantly being disturbed, especially in the small intestine; and not only the number of bacteria but bacterial diversity is lower in the small intestine. However, estimates of bacterial diversity in the colon rarely exceed 1,000 species. From a nutritional competition perspective, high diversity in an established ecosystem is thought to inhibit invasion by new species, whether they are pathogens or beneficial microorganisms. In environmental ecosystems, the low susceptibility to invasion by high-diversity communities is due to low levels of available resources, which is because redundancy of species utilization of resources reduces the niche width. Disturbances in the ecosystem may allow invading species to overcome resource-dependent limitations to invasion (Tilman 2004; Xavier et al. 2005). In complex ecosystems, superior competitors for a nutrient may be limited by growth rate; thus, they may be unable to exploit all of the available space, and inferior competitors can exploit these gaps if they have high growth rates (Amarasekare et al. 2004). Lactic acidosis in cattle that were rapidly switched from a forage diet to a high grain diet is a good example. *Streptococcis bovis*, which is normally a minor species in the rumen, has poor affinity for carbohydrates but can grow rapidly when carbohydrates are available. *S. bovis* produces lactic acid, which rapidly decreases ruminal pH, inhibiting the normal dominant microbiota. There are a variety of ecosystems in the intestinal tract, and each exerts different ecological pressures on microbial colonization. Another example would be the growth rate or metabolic activity of different sections of the intestinal tract. Although the number of bacteria and bacterial species is lower in the small intestine, if one calculates the ATP per bacterial cell in each location, the metabolic activity of the microbiota in the small intestine is tenfold greater than that of the colon (Patterson, unpublished, adapted from Jensen and Jorgensen 1994).

The conceptual framework of microbial ecological theory may help explain the different enterotypes discussed by (Arumugam et al. 2011 and Yin et al. 2010). Additional concepts for host contribution to species/enterotype colonization (e.g., differential gene expression resulting in unique epithelial cell composition/secretions, mucosal immune status) need to be developed for host/microbiota ecological theory development.

1.1   Temporal Colonization of the Intestinal Tract

The intestinal tract is sterile at birth and becomes colonized in a series of successional steps (Dominguez-Bello et al. 2011; Ley et al. 2006; Koenig et al. 2011; Lu et al. 2003; Yin et al. 2010). Facultative microorganisms rapidly colonize the intestinal tract; and as they modulate the nutritional and environmental (oxygen, pH, host gene expression) ecosystems of the intestinal tract, more anaerobic microorganisms sequentially colonize it (Dominguez-Bello et al. 2011; Koenig et al. 2011; Ley et al. 2006; Wilkinson 2002). The early, rapid initial colonization helps protect against pathogen invasion (colonization resistance), but the climax microbial population may not become established for several years or even until the adolescent period in an animal (Marques et al. 2010). Colonization is dependent on the microorganisms in the host animal's environment, the host's physiology, and the host animal's response to the early colonizers. In adult animals, there is a gradient of oxygen from food and water consumption and a gradient from tissues into the lumen that influences species composition along the digestive tract (Wilkinson 2002). There is an increase in total numbers of bacteria along the small intestinal tract, in the cecum, and in the proximal and distal large intestine resulting in facultative microorganism being <0.01–1.0% of the total population. The ecosystems influence the types of microorganism colonizing each ecosystem and are the source of microorganisms that colonize subsequent intestinal ecosystems (see Rawls et al. 2006 and Yin et al. 2010 for a discussion of the impact of the microbiota source and the host on microbial colonization). The stability of the intestinal microbiota also changes in the elderly (Claesson et al. 2011; Spor et al. 2011).

1.2   Postnatal Programming

There is increasing information about fetal and postnatal programming, not only regarding behavior (Heijtz et al. 2011; Huang 2011; Li et al. 2009; Marques et al. 2010; Tarry-Adkins and Ozanne 2011) but also about metabolic and immune function (Badr and Mohany 2011; Leach and Mann 2011). The most detailed example of postnatal programming interactions with the microbiota was offered by Hooper et al. (1999). They described the programmed expression of fucose on epithelial cell surfaces in germ-free mice during early postnatal development and demonstrated

that fucose expression in germ-free mice lasted only briefly. However, in the presence of *Bacteroides thetaiaotamicron*, fucose expression expanded among epithelial cells and continued in the presence of *B. thetaiotamicron*. They also showed that *B. thetaiotamicron* secreted a signal molecule stimulating expression of fucose, which the bacterium could use as a nutrient source. It would be naive to assume that this was the only molecule in the intestinal tract with programmed expression, with subsequent regulation by the presence of bacterial signals.

Another example of the importance of early colonization by specific microbial populations is that children who develop allergies are colonized less frequently with bifidobacteria and enterococci but more frequently with *Clostridium difficile*; moreover, there is a correlation between nonallergic children and secretory immunoglobulin A (SIgA) levels. Early colonization with bifidobacterium species correlated with higher levels of SIgA in Swedish infants 1 month after birth, whereas there was a negative correlation between *Bacteroides fragilis* and toll-like receptor 4 (TLR4), CCL4, and interleukin-6 (IL-6) expression in peripheral mononuclear cells 12 months after birth (Sjögren et al. 2009).

The initial inoculum is primarily thought to be bacteria from the mother's rectal/vaginal microbiota, although other environmental sources may also be important. Tapiainen et al. (2006) showed that the gut microbiota varied significantly over the first few days of life and varied among individuals. The fecal microbiota resembled that of both the mothers and their nurses 6 months after birth. Other factors influencing the microbiota include mode of delivery, gestational age, antibiotic use, hospitalization, surrounding environment, and maternal infection (Adlerberth and Wold 2009; Marques et al. 2010; Tanaka et al. 2009). We (Patterson, unpublished data) have shown that chickens raised intensively versus on pasture carry *Salmonella* longer in the cecum but not the ileum. Yin et al. (2010) used two continuous culture inocula or material from adult birds or water to inoculate day-old chicks. Both continuous culture inocula contained ~36% *Bacteroides*, 61% *Firmicutes*, and 3% *Proteobacteria*; however, one inoculum was characterized by much higher levels of *Bacteroides fragilis*, whereas the second inoculum contained much higher levels of *Prevotgella albensis*, *Acidaminococcus*, and *Dorea*. Over 15 days, the chicks developed significantly different microbial populations, with the latter treatment and water inoculum having more similar populations. Gene expression in ileal samples was also different between the three inocula. Thus, certain microbial populations inoculated early have lasting effects on the subsequent microbial populations.

Early life exposure to microbes drives expansion and development of immune cells and tissues; and the diversity and specific types of microorganisms at least partially influence subsequent ability of the immune system to respond to allergens and infection (Bjorksten et al. 2001). Exposure of neonatal piglets to low- and high-hygiene environments showed a greater diversity of microbiota in low-hygiene-raised piglets, slower accumulation of dendritic cells in the intestinal mucosa, and differential production of IL-2 and IL-4 by mucosal and systemic T cells (Inman et al. 2010). Arumugam et al. (2011) found three enterotypes among humans from different cultures characterized by high levels of either *Bacteroides*, *Prevotella*, or *Ruminococcus* species that differ in functional properties. Hydrogen disposal may

also be a factor in enterotype development as the high *Bacteroides* enterotype is associated with higher levels of *Desulfovibrio*, and the high *Ruminococcus* enterotype is associated with higher levels of *Methanobrevibacter*. The newer high throughput molecular approaches may be able to identify other minor components of the microbiota that have important functions in the intestinal tract. Current information is not detailed enough to determine the host properties that dictate the different enterotypes and that may be influenced by host immune modulation (which may be influenced by early colonizers) and/or physiological factors such as transit time, dry matter content, or luminal pH. However (Spor et al. 2011) indicated some host genes associated with specific microbiota.

1.3 Impact of Stressors on the Intestinal Microbiota

It is well known that stress makes animals more susceptible to infection; and until recently it was thought that stress hormones acting through the HPA increased susceptibility through suppression of the immune system. Recent data show that catecholamines do increase growth and expression of virulence genes in gram-pathogenic bacteria (Freestone et al. 2008; Lyte 2004; Lyte et al. 2011). The central thesis from this group is that the intestinal microbiota is an organ, and the microbial species composition influences host homeostasis and disease susceptibility. Also, the host's nervous system (in conjunction with the immune system) influences the species composition, and the microbiota has its own nervous system (quorum sensing as well as the ability to sense and secrete a variety of signal compounds) (Lyte 2010). Massive release of norepinephrine caused by administration of a neurotoxin has been shown to cause a several-log-fold increase in *E. coli* within hours (Lyte and Bailey 1997). Bailey et al. (2010, 2011) have shown that mild stressor exposure disrupts the commensal microbial population and that infection levels were associated with the presence of specific microbial genera.

1.4 Conclusions

The picture that is emerging is that the sterile intestine is rapidly colonized by a succession of bacterial species and then slowly approaches its climax population with increasing numbers and diversity of bacteria. The composition of this early microbiota has implications regarding programming not only of the climax microbiota but also the development of the intestinal epithelium, immune system, and brain. Furthermore, this early developmental programming may influence how these systems respond to stress and disease during adulthood. The data also suggest that although there are unique individual differences in microbial composition, the developmental and climax microbial populations may be manipulated to enhance animal well-being and resistance to stress and disease. The intestinal microbiota
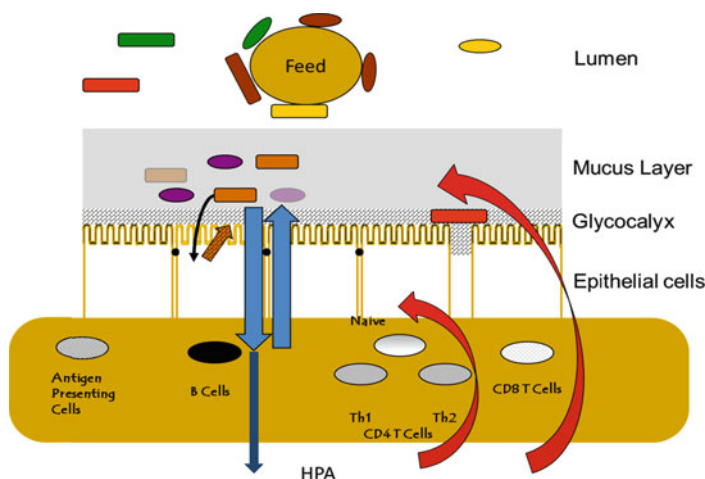
Fig. 1.1 Beneficial and pathogenic microorganisms secrete signal molecules that modulate secretion and cytoskeleton rearrangement of epithelial cells. Epithelial cells signal both luminal bacteria and mucosal immune cells, which in turn can signal the hypothalamic–pituitary axis (HPA). Signals from the HPA also influence mucosal immune cell and epithelial cell function and can influence the microbiota in the lumen of the intestinal tract

secrete signals that affect the epithelium, mucosal immune system, and brain. In turn the epithelium, mucosal immune system, and brain influence the composition of the intestinal microbiota. The interactions between these systems during development and homeostasis dictate how these systems respond to stressors and infection (Fig. 1.1).

Recent data suggest that the early microbial colonizers influence the development of subsequent microbial populations as well as host development and function. There is increasing interest in the use of probiotics, prebiotics, and other dietary additions to improve animal health and well-being. Although the efficacy of these dietary additions is variable, it may be important to address offering these dietary additions shortly after birth (Bezirtzoglou and Stavropoulou 2011; Nava et al. 2005).

References

Adlerberth, I., and A.E. Wold. 2009. Establishment of the gut microbiota in Western infants. *Acta Paediatrica* 99: 229–238.

Amarasekare, P., M.F. Hoopes, N. Mouquet, and M. Holyoak. 2004. Mechanisms of coexistence in competitive metacommunities. *The American Naturalist* 164: 310–326.

Arumugam, M., J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D.R. Mende, G.R. Fernandes, J. Tab, T. Bruls, J.M. Batto, M. Bertalan, N. Borruel, F. Casellas, L. Fernandez, L. Gautier, T. Hansen,

M. Hattori, T. Hayashi, M. Kleerebezem, K. Kurokawa, M. Leclerc, F. Levenez, C. Manichanh, H.B. Nielsen, T. Nielsen, N. Pons, J. Poulain, J. Quin, T. Sicheritz-Ponten, S. Tims, D. Torrents, E. Ugarte, E.G. Zoentendal, J. Wang, F. Guarner, O. Pedersen, W.M. de Vos, S. Brunak, J. Dore, M. Antolin, F. Artiguenave, H.M. Blottiere, M. Almedia, C. Brechot, C. Cara, C. Chervaux, A. Cultrone, C. Delorme, G. Denariaz, R. Dervyn, K.U. Foerstner, C. Friss, M. van de Guchte, E. Guedon, F. Haimet, W. Huber, J. van Hylckama-Vleig, A. Jamet, C. Juste, G. Kaci, J. Knol, O. Lakhdari, S. Layec, K. Le Roux, E. Maguin, A. Meriwux, R. Melo Minardi, C. M'Rini, J. Muller, R. Oozeer, J. Parkhill, P. Renault, M. Rescigno, N. Sanchez, S. Sunagawa, A. Torrejon, K. Turner, G. Vandemeulebrouck, E. Varela, Y. Winogradsky, G. Zeller, J. Weissenbach, S.D. Erlich, and P. Bork. 2011. Entrotypes of the human gut microbiome. *Nature* 473: 174–180.

Badr, G., and M. Mohany. 2011. Maternal perinatal undernutrition attenuates T-cell function in adult male rat offspring. *Cellular Physiology and Biochemistry* 27(3–4): 381–390.

Bailey, M.T., S.E. Dowd, N.M.A. Parry, J.D. Galley, D.B. Schauer, and M. Lyet. 2010. Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by *Citrobacter rodentium*. *Infection and Immunity* 78: 1509–1519.

Bailey, M.T., S.E. Dowd, J.D. Galley, A.R. Hufnagle, R.G. Allen, and M. Lyte. 2011. Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity* 25: 397–407.

Bezirtzoglou, E., and E. Stavropoulou. 2011. Immunology and probiotic impact of the newborn and young children intestinal microflora. *Anaerobe*. doi:10.1016/j.anarobe.2011.03.010.

Borksten, B., E. Sepp, K. Julge, T. Voor, and M. Kikelsaar. 2001. Allergy development and the intestinal microflora during the first year of life. *The Journal of Allergy and Clinical Immunology* 108: 516–620.

Claesson, M.J., S. Cusack, O. O'sullivan, R. Green-Diniz, H. de Weerd, E. Flannery, J.R. Marchesi, D. Falushy, T. Dinan, G. Fitzgerald, C. Stanton, D. van Sinderen, M. O'Conner, N. Harnedy, K. O'Conner, C. Henry, D. O'Mahony, A.P. Fitzgerald, F. Shanahan, C. Twomey, C. Hill, R.P. Ross, and P.W. O'Toole. 2011. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences of the United States of America* 108: 4586–4591.

Dominguez-Bello, M.G., M.J. Blaser, R.E. Ley, and R. Knight. 2011. Development of the human gastrointestinal microbiota and insights from high-throughput sequencing. *Gastroenterology* 140: 1713–1719.

Freestone, P.P.E., S.M. Sandrini, R.D. Haigh, and M. Lyte. 2008. Microbial endocrinology: How stress influences susceptibility to infection. *Trends in Microbiology* 16: 55–64.

Gill, S.R., M. Pop, R.T. Deboy, P.B. Eckburg, P.J. Turnbaugh, B.S. Samuel, J.I. Gordon, D.A. Relman, C.M. Fraser-Liggett, and K.E. Nelson. 2006. Metagenomic analysis of the human distal gut microbiome. *Science* 312: 1355–1359.

Heijtz, R.D., S. Wang, F. Anuar, Y. Qian, B. Björkholm, A. Samuelsson, M.L. Hibberd, H. Forssberg, and S. Pettersson. 2011. Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America* 108: 3047–3052.

Hooper, L.V., J. Xu, P.G. Falk, T. Midtvedt, and J.I. Gordon. 1999. A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. *Proceedings of the National Academy of Sciences of the United States of America* 96: 9833–9838.

Horner-Devine, M.C., K.M. Carney, and B.J.M. Bohannan. 2004. An ecological perspective on bacterial biodiversity. *Proceedings of the Royal Society of London Series B* 271: 113–122.

Huang, L.T. 2011. The link between perinatal glucocorticoids exposure and psychiatric disorders. *Pediatric Research* 69(5 Pt 2): 19R–25R.

Inman, C.F., K. Haverson, S.R. Konstantinov, P.H. Jones, C. Harris, H. Smidt, B. Miller, M. Bailey, and C. Stokes. 2010. Rearing environment affects development of the immune system in neonates. *Clinical and Experimental Immunology* 160: 431–439.

Jensen, B.B., and H. Jorgensen. 1994. Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. *Applied and Environmental Microbiology* 60: 897–904.

Koenig, J.E., A. Spor, N. Scalfone, A.D. Fricker, S. Stombaugh, R. Knight, L.T. Angenet, and R.E. Lay. 2011. Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America* 108: 4578–4585.

Leach, L., and G.E. Mann. 2011. Consequences of fetal programming for cardiovascular disease in adulthood. *Microcirculation* 18(4): 253–255.

Ley, R.E., D.I. Pederson, and J.I. Gordon. 2006. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124: 837–848.

Li, W., S.E. Dowd, B. Scurlock, V. Acosta-Martinez, and M. Lyte. 2009. Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. *Physiology and Behavior* 96: 557–567.

Lu, J., U. Idris, B. Harmon, and C. Hofacre. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Applied and Environmental Microbiology* 69: 6816–6824.

Lyte, M. 2004. Microbial endocrinology and infectious disease in the 21st century. *Trends in Microbiology* 12: 14–20.

Lyte, M. 2010. The microbial organ in the gut as a driver of homeostasis and disease. *Medical Hypotheses* 74: 634–638.

Lyte, M., and M.T. Bailey. 1997. Neuroendocrine-bacterial interactions in an neurotoxin-induced model of trauma. *The Journal of Surgical Research* 70: 195–201.

Lyte, M., L. Vulchanova, and D.R. Brown. 2011. Stress at the intestinal surface: Catecholamines and mucosa–bacteria interactions. *Cell and Tissue Research* 343: 23–32.

Marques, T.M., R. Wall, R.P. Ross, G.F. Fitzgerald, C.A. Ryan, and C. Stanton. 2010. Programming infant gut microbiota: Influence of dietary and environmental factors. *Current Opinion in Biotechnology* 21: 149–156.

Nava, G.M., L.R. Bielke, T.R. Callaway, and M.P. Castañeda. 2005. Probiotic alternatives to reduce gastrointestinal infections: The poultry experience. *Animal Health Research Reviews* 6: 105–118.

O'Hara, A.M., and F. Shanahan. 2006. The gut flora as a forgotten organ. *EMBO Reports* 7: 688–693.

Rawls, J.F., M.A. Mahowald, R.E. Ley, and J.I. Gordon. 2006. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. *Cell* 127: 423–433.

Sjögren, Y.M., S. Tomicic, A. Lundberg, M.F. Böttcher, B. Björkstén, E. Sverremark-Ekström, and M.C. Jenmalm. 2009. Infulence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. *Clinical and Experimental Allergy* 39: 1842–1851.

Spor, A., O. Koren, and R.E. Ley. 2011. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology* 9: 279–290.

Tanaka, S., T. Kobayashi, P. Songjinda, A. Taeyama, M. Tsucouchi, C. Kiyohara, T. Shirakawa, K. Sonomoto, and J. Nakayama. 2009. Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. *FEMS Immunology and Medical Microbiology* 56: 80–87.

Tapiainen, T., S. Ylitalo, E. Eerola, and M. Uhari. 2006. Dynamics of gut colonization and sources of intestinal flora in healthy newborn infants. *APMIS* 114: 812–817.

Tarry-Adkins, J.L., and S.E. Ozanne. 2011. Mechanisms of early life programming: Current knowledge and future directions. *The American Journal of Clinical Nutrition*. doi:10.3945/ajcn.110.000620.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: A stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences of the United States of America* 101: 10854–10861.

Wilkinson, M.H.F. 2002. Model intestinal microflora in computer simulation: A simulation and modeling package for host-microflora interactions. *IEEE Transactions on Biomedical Engineering* 49: 1077–1085.

Xavier, J.B., C. Picioreanu, and M.C. van Loosdrecht. 2005. A framework for multidimensional modelling of activity and structure of multispecies biofilms. *Environmental Microbiology* 8: 1085–1103.

Yin, Y., F. Lei, L. Zhu, S. Li, Z. Wu, R. Zhang, G.F. Gao, B. Zhu, and X. Wang. 2010. Exposure of different baco newborn chicken affects gut microbiota development and ileum gene expression. *The ISME Journal* 4: 367–376.

Chapter 2
Prebiotics of Plant and Microbial Origin

Brittany M. Vester Boler and George C. Fahey Jr.

Abstract The food industry is constantly shifting focus based on what is most important to the consumer. Products are marketed currently that are believed to provide health benefits to the consumer such as beneficial effects on health or as disease preventatives. Much of the focus is on oligosaccharides as health-promoting substrates. Many oligosaccharides are resistant to digestion and absorption by mammalian enzymes and, therefore, reach the large bowel where they may be fermented by the resident bacteria. Beyond their potential as substrates for fermentation, oligosaccharides are popular food additives due in large part to their low caloric value and their ability to enhance mineral absorption. Health benefits include alleviation of constipation, reduced risk of infection and diarrhea, and improved immune response. Many oligosaccharides modulate microbiota of the large bowel by increasing bifidobacteria and lactobacilli populations and decreasing clostridia populations. This review will describe the manufacturing processes for select non-digestible oligosaccharides and other food ingredients currently classified as prebiotics and those with prebiotic potential.

2.1 Introduction

The food industry is constantly shifting focus based on what is most important to the consumer. Products are marketed currently that are believed to provide health benefits to the consumer. They are touted as either having beneficial effects on health or as disease preventatives. Because of the increased demand for these types of product, there is a growing interest in this research area that not only helps food

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companies make accurate claims on products but helps identify new products that may lead to enhanced health.

Much of the focus is on oligosaccharides as health-promoting substrates. Oligosaccharides are low-molecular-weight carbohydrates with a low degree of polymerization (DP). They are either 2–20 monosaccharide units or no more than 10 monosaccharide units, depending on the official definition used (IUP-IUPAC 1982; Food and Drug Administration 1993). Many oligosaccharides are resistant to digestion and absorption by mammalian enzymes; and they therefore reach the large bowel, where they may be fermented by the resident bacteria. These oligosaccharides cannot be digested because the anomeric carbon atom has a configuration making the osidic bond resistant to mammalian enzymes. These oligosaccharides are termed "nondigestible oligosaccharides" (NDO).

Beyond their potential as substrates for fermentation, NDO are popular food additives due in large part to their low caloric value and their ability to enhance mineral absorption. NDO are water-soluble and sweet tasting; however, the sweetness decreases with increasing chain length. These products can aid in water binding and gelling, which can potentially decrease the amount of fat needed in a food product (Roberfroid and Slavin 2002). Health benefits linked to NDO ingestion include alleviation of constipation (Marteau 2001; Kaur and Gupta 2002), less risk of infection and diarrhea (Mussatto and Mancilha 2007), and improved immune response (Jenkins et al. 1999; Kelly-Quagliana et al. 2003; Manning and Gibson 2004). Other potential health benefits include modulation of lipid metabolism, reduced cancer risk, and treatment of hepatic encephalopathy (Swennen et al. 2006; Mussatto and Mancilha 2007). Many NDO also beneficially modulate microbiota of the large bowel by increasing bifidobacteria and lactobacilli populations and decreasing clostridial populations.

Select NDO have been classified as prebiotics. The concept of prebiotics was first introduced in 1995 (Gibson and Roberfroid 1995), and they have gained attention in industry and academia due to their potential health benefits. Prebiotics are defined as nondigestible food ingredients that are resistant to digestion and absorption (nondigestible), are fermented by cecal/colonic microbiota, and selectively stimulate growth and/or activity of bacteria that contribute to colonic and host health (Gibson et al. 2004; Roberfroid 2007). Only three NDO to date can be definitively classified as prebiotics: fructans, galactooligosaccharides (GOS), and lactulose. Although other NDO may have prebiotic potential, only limited research is available or the current data are conflicting, which does not allow them to be termed prebiotics. This review describes the manufacturing processes for select NDO and other food ingredients currently classified as prebiotics and those with prebiotic potential.

2.1.1   Methods of Manufacture

Nondigestible oligosaccharides have many uses in the food industry beyond use as a prebiotic. They have been used in food products to add bulk, reduce sweetness

Table 2.1 Definitions of some processing terms

Term	Definition
Hydrolysis	The cleaving of a molecule into two parts with the addition of a molecule of water
Extraction	Separation of compounds based on their solubility in two different liquids (usually water and an organic solvent)
Isomerization	The transformation of one molecule into a different one with the same molecular formula, but with a different structure
Transglycosylation	The transfer of a sugar residue from one glycoside to another

when other flavors should predominate, mask the taste of artificial sweeteners, and improve the mouth feel owing to their viscosity properties (Crittenden and Playne 1996; Mussatto and Mancilha 2007). Just as important, NDO are generally classified as "generally recognized as safe" (GRAS) and can be added to products meant for human and animal consumption. These products are generally safe and lead to only transient side effects when consumed in large doses; however, what constitutes a large dose is person/animal-dependent. Side effects of fermentable NDO include severe flatulence, intestinal discomfort, and osmotic diarrhea (Pederson et al. 1997; Cummings et al. 2001; Marteau 2001; Juśkiewicz and Zduńczyk 2002).

There are three main manufacturing processes for NDO: direct extraction from plants; controlled enzymatic hydrolysis of high-DP polysaccharides to lower DP oligosaccharides; and enzymatic-catalyzed synthesis via microbial action on simple sugars (Grizard and Barthomeuf 1999; L'Hocine et al. 2000). These processes use various chemical reactions, defined in Table 2.1. Inulin and soybean oligosaccharides (raffinose and stachyose) are two examples of NDO that can be directly extracted from plant sources (chicory root and soybeans, respectively). Commercially produced inulin, however, also undergoes hydrolysis of longer-chain polysaccharides to create a final product. Another example of controlled hydrolysis includes production of xylooligosaccharides from xylan. Finally, others are built using transglycosylation reactions with simple sugars such as the production of lactulose (Prenosil et al. 1987; Nilsson 1988; Okazaki et al. 1990; Playne 1994; Crittenden and Playne 1996).

2.1.2   *Manufacture of Established Prebiotics*

Fructans include inulin-type and levan-type oligosaccharides. Inulin-type fructans have β-2,1-ᴅ-fructofuranosyl units, are found in plants and synthesized by fungi, and have a DP of 2–70. Levan-type fructans have β-6,2-ᴅ-fructofuranosyl units, are found in plants and synthesized by bacteria, and have a DP > 30. Fructans occur naturally in plants such as chicory, Jerusalem artichoke, dahlia, salsify, gobo, onion, garlic, leek, and wheat by-products; and they serve as an energy source for these plants. Only inulin-type fructans are proven prebiotics (Roberfroid et al. 1998).
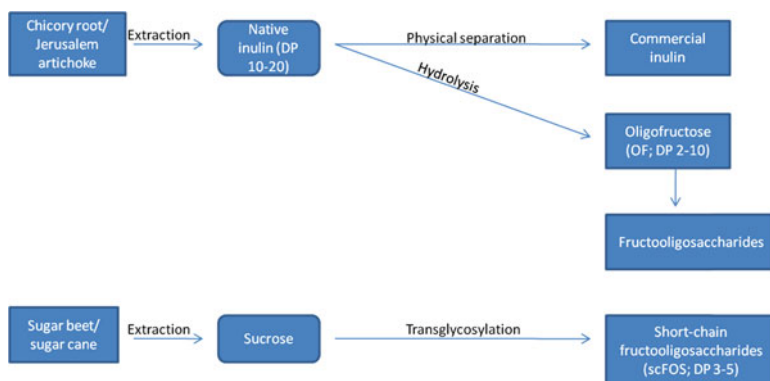
Fig. 2.1 Commercial production of inulin-type fructans from extracts of natural sources, partial enzymatic hydrolysis, or enzymatic synthesis from sucrose. *DP* degree of polymerization

Inulin is manufactured through direct hot water extraction from natural sources, mainly chicory root (Debruyn et al. 1992). It is composed of β(2-1) linkages of glucose and fructose [$G_{py}F_n$: α-D-glucopyranosyl-(β-D-fructofuranosyl)$_{n-1}$–D-fructofuranoside] or only fructose [$F_{py}F_n$: β-D-fructopyranosyl-(α-D-fructofuranosyl)$_{n-1}$–D-fructofuranoside] (Roberfroid and Delzenne 1998). Between 2 and 70 units of fructose may be present in native inulin, and it has an average DP of 10–20. Inulin comprises 15–20% of chicory root fresh weight with 55% of oligosaccharides with a DP of 2–19, 28% with a DP of 20–40, and 17% with a DP of >40. It comprises 17–20% of Jerusalem artichoke fresh weight with 74% of oligosaccharides with a DP of 2–19, 20% with a DP of 20–40, and 6% with a DP of >40 (Van Loo et al. 1995).

After extraction of native inulin, the product then undergoes either industrial physical separation of long-chain fructans (De Leenheer 1996) or is partially hydrolyzed by endoinulinase to produce short-chain oligosaccharides, mainly oligofructose (Fig. 2.1). Oligofructose produced from inulin may or may not have a terminating glucose molecule, may contain longer-chain fructans (Crittenden and Playne 1996), and has a DP of 2–10 (average 5) (Roberfroid and Delzenne 1998). Alternatively, short-chain fructooligosaccharides can be produced synthetically through transfructosylation of sucrose using the β-fructofuranosidase enzyme (Crittenden and Playne 1996) from *Aureobasidium pullulans* (Yun 1996; Yoshhikawa et al. 2008) or *Aspergillus niger* (Park and Almeida 1991). These compounds contain 2–4 fructosyl units with a terminal glucose unit and an average DP of 3.5 (Roberfroid and Delzenne 1996). Synthetic fructooligosaccharides contain only $G_{py}F_n$ oligomers. These products may contain free glucose, fructose, and sucrose, which can be removed via chromatographic procedures to increase the purity of the final product. It should be noted, however, that a large amount of starting material is needed to achieve efficient transglycosylation (Park and Almeida 1991).

Fructans are perhaps the most well-established prebiotics (Roberfroid 2007) and the most extensively studied. They meet the three key criteria defining a prebiotic,
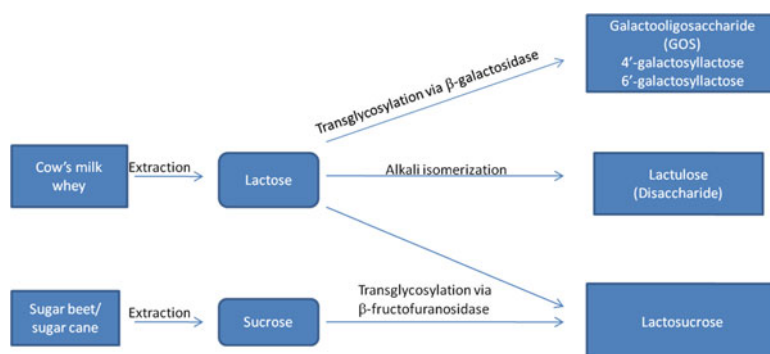
Fig. 2.2 Commerical production of lactose-derived prebiotics via transglycosylation to produce galactooligosaccharides, alkali isomerization to produce lactulose, or transglycosylation with sucrose to produce lactosucrose

that inulin-type fructans are nondigestible (Cherbut 2002), are fermented in the large bowel, and lead to selective growth of bacteria associated with health in vitro (Roberfroid et al. 1998) and in vivo [human subjects, including infants (Coppa et al. 2002), adults (Harmsen et al. 1999), and the elderly (Guigoz et al. 2002)].

Galactooligosaccharides are produced from lactose (Fig. 2.2) and are defined as oligosaccharides with 2–8 galactose or disaccharide units (two units of galactose) with a terminal glucose. Commercial production utilizes highly concentrated lactose from cow milk whey. Lactose undergoes transglycosylation from β-galactosidase enzymes (the glycosyltransferases and glycohydrolases). The types of GOS produced depend on the type of β-galactosidases used and the processing conditions (Mussatto and Mancilha 2007).

Galactosyltransferases move a sugar unit from the donor to the receptor molecule, forming a glycosidic bond (Tzortis and Vulevic 2009). Although production of GOS is relatively efficient, galactosyltransferase enzymes are difficult to find; and the need for sugar nucleotides in the reaction makes them cost-prohibitive to the industry. Galactohydrase enzymes are more readily available but lack the specificity of galactosyltransferases. Microbial stains used for enzyme production include *A. oryzae* and/or *Strep. thermophilus*, which form β-1,6 bonds, or *Bacillus circulans* or *Cryptococcus laurentii*, which form β-1,4 bonds (Sako et al. 1999).

Approximately 55% of the starting lactose is converted to GOS. These GOS produced are mostly trisaccharides (4′-galactosyllactose and 6′-galactosyllactose) that have 2–5 galactose units and longer-chain oligosaccharides with four or more monosaccharide units. Generally, 80% of the oligosaccharides formed are trisaccharides (Playne and Crittenden 2004). Other products present at the end of the reaction include lactose, galactose, and glucose disaccharides and transgalactosylated disaccharides (Sako et al. 1999). These transgalactosylated disaccharides produced are considered NDO as they have properties similar to those of longer-chain GOS.

Commercial GOS products are generally made in batch systems for simplicity; however, this method is the least efficient, and most of the enzyme added to the initial reaction is lost. Continuous systems have been proposed to cut production costs by using ultrafiltration to retain soluble enzymes via enzyme immobilization (Tzortis and Vulevic 2009). During batch reactions, multiple enzymes may be added at the initial reaction or in sequence during the reaction. The mixture is heated to facilitate lactose solubilization and drive the formation of oligosaccharides over hydrolysis of lactose to monosaccharides (Playne and Crittenden 2004). Unreacted products may be removed using chromatography, although lactose has been noted to be difficult to remove and leads to a loss of GOS. Furthermore, the product is decolorized, demineralized, and concentrated to a syrup or powder form. A highly consistent product can be developed by maintaining strict production conditions, although all final products are mixtures of various GOS products (Playne and Crittenden 2004).

Although there is no overwhelming evidence of its prebiotic potential, as is the case with fructans, GOS are considered to be a proven prebiotic (Roberfroid 2007). Evidence, though minimal, suggests that GOS are not hydrolyzed by mammalian enzymes (Tanaka et al. 1983). It has been established that selective bacterial stimulation occurs, however, as increases in bifidobacteria and lactobacilli have been noted in several studies (Ito et al. 1990; Bouhnik et al. 1997; Moro et al. 2002). Therefore, although it is probable that GOS are not digested by mammalian enzymes and it has been proven that they lead to selective growth of beneficial bacteria, few data are available on their fermentation potential in the large bowel (Roberfroid 2007).

Lactulose, like GOS, is produced from lactose (Fig. 2.2). It is formed through alkali isomerization of the glucose moiety of lactose to fructose, thereby making it a combination of fructose and galactose. The resulting disaccharides with β-1,4 linkages are not digested by mammalian enzymes. To manufacture lactulose, lactose is mixed with an alkali (e.g., sodium hydroxide), and a catalyst may be added (Playne and Crittenden 2004). The mixture then is heated to facilitate isomerization. The unreacted lactose is removed, and the product is pasteurized and then concentrated into syrups, powders, or crystals.

Commercial lactulose is expensive to produce, as only 20–30% of lactose is converted after isomerization, and expensive purification techniques are required. Lactulose is known to have prebiotic effects and may be used in that capacity or as a low-calorie sweetener. Most lactulose (90%), however, is used pharmaceutically to prevent constipation and in patients with hepatic encephalopathy to reduce blood ammonia concentrations (Crittenden and Playne 1996).

Despite the fact that lactulose is classified as a proven prebiotic because of its extensive published human database, its use as a food supplement is limited (Roberfroid 2008). This is probably because lactulose is resistant to mammalian enzymes (Gibson and Angus 2008), although research on the subject is limited. Key animal and human studies indicate that it is fermented in the large bowel, and it has the ability selectively to stimulate bifidobacteria and lactobacilli populations (Terada et al. 1993; Ballongue et al. 1997; Tuohy et al. 2002). Therefore, although further research would help clarify its status, lactulose is considered a proven prebiotic (Roberfroid 2007, 2008).
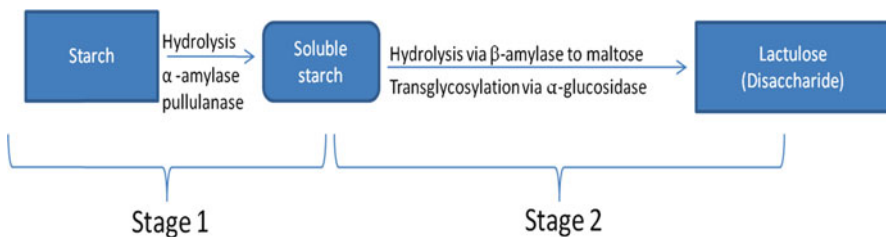
Fig. 2.3 Commercial production of lactulose, a two-stage process. The first stage hydrolyzes starch to a liquefied product. During the second phase, soluble starch undergoes transglycosylation to lactulose

2.1.3 Manufacture of Promising Prebiotics

Isomaltooligosaccharides (IMO) consist of glucose monomers with α-1,6 glucosidic bonds (Fig. 2.3). Although the food industry uses commercially produced material, IMO occur naturally in miso, soy sauce, sake, and honey. Commercial production of IMO is a two-stage process, with starch as the starting material. Starch is first hydrolyzed with α-amylase and pullulanase to make a liquefied starch product. β-Amylase hydrolyzes the liquefied starch to maltose, and then the transglucosidase activity of α-glucosidase produces IMO with a maximum final concentration of 40% of the total mixture (Casci and Rastall 2006). Unreacted glucose (approximately 40% of the final mixture) is then removed, and the product is concentrated. Although in vitro and cell culture data indicate that IMO have potential as prebiotics, to date limited data are available to classify it as such.

Lactosucrose is a trisaccharide produced from lactose and sucrose in a reversible reaction (Fig. 2.2). The fructosyl moiety of sucrose forms a β-2,1 glycosidic bond to the glucose residue of lactose to create lactosucrose. This is done by transglycosylation via a β-fructofuranosidase enzyme, which produces a nonreducing oligosaccharide (Hara et al. 1994). This enzyme, however, also can hydrolyze sucrose (Mussatto and Mancilha 2007). Therefore, in a batch system, with an initial equimolar ratio, there is a yield of only 52% lactosucrose (Kawase et al. 2001). Cleanup of this product is complicated and includes decolorization, filtration, concentration, purification, filtration, deionization, and, again, concentration (Playne and Crittenden 2004). Although lactosucrose has been noted to be bifidogenic in small human trials (Kumemura 1992; Ohkusa et al. 1995), the data regarding its bifidogenic capabilities are limited. Moreover, there are no data available on its ability to withstand hydrolysis in the gastrointestinal tract (Roberfroid 2008).

Xylooligosaccharides (XOS) consist of chains of xylose molecules with β-1,4 linkages. It is a naturally occurring oligosaccharide found in honey, bamboo shoots, fruits, vegetables, and milk (Vázquez et al. 2000). It also can be made by breaking down the polysaccharide, xylan, a major component of hemicelluloses, into XOS. Commercial production is conducted through enzymatic hydrolysis of primarily corncobs but also straws, hardwoods, bagasse, hulls, and bran using endo-1,4-β-xylanase

Fig. 2.4 Commercial production of xylooligosaccharides, which are most commonly from corncobs but also can be extracted from hardwood. Xylooligosaccharides are extracted by hydrolyzing xylans in the starting material using endo-1,4-β-xylanase

(Fig. 2.4). Using enzymes with low exoxylanase and/or β-xylosidase activity is prudent to minimize production of xylose. Xylanases are produced by *Trichoderma reesei*, *T. harzianum*, *T. viride*, *T. koningii*, and *T. longibrachiatum* (Chen et al. 1997; Casci and Rastall 2006). Other methods for extracting XOSs include (1) chemical fractionation of material to isolate xylan with further enzymatic hydrolysis and (2) hydrolytic degradation of xylan by steam, water, or dilute mineral acid solutions (Vázquez et al. 2000). Chains produced through extraction in all processes include xylobiose, xylotriose, and xylotetraose (Hopkins et al. 1998).

Prior to cleaning, the solution contains approximately 60–70% XOS (Playne and Crittenden 2004). After production, xylose and other compounds are removed with ultrapurification and reverse osmosis (Crittenden and Playne 1996) to create a product generally containing 70% or 95% oligosaccharides (Xylooligo 70 and Xylooligo 95, respectively) (Playne and Crittenden 2004). Data indicate that XOS are bifidogenic (Okazaki et al. 1990; Campbell et al. 1997). It is likely that they are resistant to hydrolytic digestion because xylan is a dietary fiber, but there are no data to support this assumption. Therefore, XOS cannot currently be classified as a prebiotic (Roberfroid 2008).

2.1.4 Manufacture of Potential Prebiotics

In addition to the previously described NDO that are considered prebiotics or potential prebiotics, there are several others that have not yet met the burden of proof to be classified as prebiotics. This is most commonly due to the limited research on these compounds. Alternatively, the limited data that do exist may show conflicting results, and some researchers have not evaluated changes in the microbiota. Beyond oligosaccharides, some longer-chain carbohydrate sources may also have prebiotic capabilities. Those potential prebiotic carbohydrates are listed in Table 2.2. There are even noncarbohydrate food ingredients that have potential to be classified as prebiotics, including lactoferrin, phenolic compounds (e.g., flavonoids), and glutamine. To date, however, little research has been done on any of those compounds.

Interestingly, polysaccharides with prebiotic potential may be more beneficial than some NDO prebiotics. This is due to the fact that polysaccharides can be consumed in higher doses without adverse side effects, such as intestinal discomfort

Table 2.2 Potential prebiotic carbohydrates

• Soybean oligosaccharides	• Mannanoligosaccharides (yeast cell wall)
• Glucooligosaccharides	• Lactose
• Cyclodextrins	• Resistant starch and derivatives
• Gentiooligosaccharides	• Oligosaccharides from melobiose
• Germinated barley foodstuffs	• N-acetylchitooligosaccharides
• Oligodextrans	• Polydextrose
• Glucuronic acid	• Sugar alcohols
• Gentiooligosaccharides	• Konjac glucomannan
• Pectic oligosaccharides	• Whole grains

and excessive flatulence (Crittenden 2006). Polysaccharides also have the potential of being fermented throughout the length of the colon, including the distal colon, the major site of large bowel disease. Potential prebiotics of interest include resistant starch, whole grains, and polydextrose.

Not all dietary starch is hydrolyzed and absorbed, as some starch is resistant to enzymatic hydrolysis. This fraction is termed resistant starch. How much starch reaches the large bowel and if it can be fermented depend on its source and structure. Resistant starch is classified in one of four ways (1) RS1, which is physically inaccessible starch (e.g., starch in whole grains); (2) RS2, which is granular starch (e.g., starch in green bananas or uncooked potatoes); (3) RS3, which is retrograded starch (e.g., starch in cooked, then cooled, potatoes); or (4) chemically modified starch (e.g., esterified starch) (Brown 1996).

Research on the prebiotic effects of resistant starch has focused on RS2 and RS3. Although research is lacking overall, RS2 and RS3 have been noted to have prebiotic capabilities in animals and animal models (Brown et al. 1997; Silvi et al. 1999; Wang et al. 2002; Dongowski et al. 2005; Jabocasch et al. 2006) and humans (Bouhnik et al. 2004). Resistant starch has been noted in several studies to increase bifidobacteria and/or lactobacilli populations (Kleessen et al. 1997; Silvi et al. 1999; Wang et al. 2002; Dongowski et al. 2005; Jabocasch et al. 2006) and to increase short-chain fatty acids, especially butyrate (Brown et al. 1997). Not all studies, however, report a bifidogenic response by resistant starches (Bird et al. 2004). Although it is evident that resistant starch is not digested by mammalian enzymes and can be fermented, the largest question remaining as to its prebiotic potential is its selectivity, as many other bacterial species are amylolytic, and many of the health benefits of resistant starch can be attributed to the production of butyrate (Crittenden 2006).

Polysaccharides are beginning to receive more attention as potential prebiotics. Furthermore, the use of whole grains in the food industry has increased markedly. Whole grains themselves have been investigated, as well as oligosaccharides that can be obtained through breakdown of polysaccharides by glyconases (Mussatto and Mancilha 2007). Limited research indicates these oligosaccharides have prebiotic potential (Rastall and Maitin 2002).

The best studied whole grain components include β-glucans and arabinoxylan oligosaccharides, which are fermented in the gastrointestinal tract (Fleming et al. 1983; Fincher and Stone 1986). In vitro studies indicate that β-glucans are not well

fermented by bifidobacteria and lactobacilli (Crittenden et al. 2002). Arabinoxylan, however, may have a bifidogenic effect and may be of particular use as it is more slowly fermented than inulin (Karppinen et al. 2000).

Whole grains have limited fermentation potential in vitro, but after processing the substrates produce greater quantities of total short-chain fatty acids (Hernot et al. 2008); however, bacterial population changes were not reported in that study. In vivo, whole grains resulted in greater *Bifidobacterium* spp. and lactobacilli concentrations in healthy adults after consuming wheat bran as a wheat bran-based breakfast cereal or whole grain as a 100% whole grain wheat cereal. This effect was greater with whole grains (Constabile et al. 2008). It is clear that whole grains and their components have potential prebiotic effects, but more research is needed regarding their fermentative capabilities and bacterial selectivity.

Polydextrose is a polysaccharide formed through acid-catalyzed vacuum thermal polymerization of glucose and sorbitol that has an average DP of 12 but can be as high as 30 (Stowell 2009; Li 2010). It is a highly branched compound, with β-1,6 linkages predominating. Polydextrose currently is used to replace fat and sucrose in food products, as a humectant, and to provide mouth feel and bulk to food products (Murphy 2001). It is well documented that it resists enzymatic hydrolysis by mammalian enzymes (Figdor and Rennhard 1981; Achour et al. 1994; Fava et al. 2007). Animal and human studies report that approximately 30–50% of polydextrose is fermented in the large bowel (Figdor and Rennhard 1981; Achour et al. 1994).

In vitro studies indicate that polydextrose enhances butyrate and other short-chain fatty acid production (Stowell 2010). There is emerging evidence that polydextrose leads to selective increases in beneficial bacteria. Jie et al. (2000) reported increased *Bifidobacterium* and *Lactobacillus* spp. in healthy adult humans, and Tiihonen et al. (2008) noted greater bifidobacteria species in healthy adults fed polydextrose plus a probiotic compared with the probiotic alone. Therefore, polydextrose likely has prebiotic capabilities, but more research is needed to determine conclusively if this is the case.

2.2 Conclusions

There are three carbohydrates proven to meet all requirements of the prebiotic definition: inulin-type fructans, GOS, and lactulose. All three prebiotics are manufactured in a unique manner but have a common characteristic in that all need a large amount of starting substrate to produce the end-product. Relatively consistent final products for use by humans and animals result from the manufacturing process.

Several other NDO show promising results in the limited research available regarding their prebiotic potential. Some prebiotics with promising results include IMO, lactosucrose, and XOS. Finally, there are even more carbohydrate and noncarbohydrate food ingredients that may have prebiotic effects when fed to animals and humans. More research defining their prebiotic potential is needed.

References

Achour, L., B. Flourie, F. Briet, et al. 1994. Gastro-intestinal effects and energy value of polydextrose in healthy non-obese men. *American Journal of Clinical Nutrition* 59: 1362–1368.

Ballongue, J., C. Schumann, and P. Quignon. 1997. Effects of lactulose and lactitol on colonic microflora and enzymatic activity. *Scandinavian Journal of Gastroenterology* 222: 41–44.

Bird, A.R., M. Jackson, R.A. King, et al. 2004. A novel high-amylose barley cultivar (*Hordeum vulgare* var. Himalaya 292) lowers plasma cholesterol and alters indices of large-bowel fermentation in pigs. *British Journal of Nutrition* 92: 607–615.

Bouhnik, Y., B. Flourié, L. D'Agay-Abensour, et al. 1997. Administration of transgalactooligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. *Journal of Nutrition* 127: 444–448.

Bouhnik, Y., L. Raskine, G. Simoneau, et al. 2004. The capacity of nondigestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel group, dose–response relation study. *American Journal of Clinical Nutrition* 80: 1658–1664.

Brown, I. 1996. Complex carbohydrates and resistant starch. *Nutrition Reviews* 54: 3115–3119.

Brown, I., M. Warhurst, J. Arcot, et al. 1997. Fecal numbers of bifidobacteria are higher in pigs fed *Bifidobacterium longum* with high amylose cornstarch than with low amylose cornstarch. *Journal of Nutrition* 127: 1822–1827.

Campbell, J.M., G.C. Fahey Jr., and B.W. Wolf. 1997. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH, and microflora in rats. *Journal of Nutrition* 127: 130–136.

Casci, T., and R.A. Rastall. 2006. Manufacture of prebiotic oligosaccharides. In *Prebiotics: Development and application*, ed. G.R. Gibson and R.A. Rastall. West Sussex, UK: Wiley.

Chen, C., J.-L. Chen, and T.-Y. Lin. 1997. Purification and characterization of a xylanase from *Trichoderma longibrachiatum* for xylooligosaccharide production. *Enzyme and Microbial Technology* 21: 91–96.

Cherbut C (2002) Inulin and oligofructose in the dietary fibre concept. *British Journal of Nutrition* 87, Suppl. 2: S159–S162.

Constabile, A., A. Klinder, F. Fava, et al. 2008. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: A double-blind, placebo-controlled, crossover study. *British Journal of Nutrition* 99: 110–120.

Coppa, G.V., S. Bruni, and L. Zampini. 2002. Prebiotics in infant formulas: Biochemical characterization by thin layer chromatography and high performance anion exchange chromatography. *Digestive and Liver Disease* 34: S124–S128.

Crittenden, R.G. 2006. Emerging prebiotic candidates. In *Prebiotics: Development and application*, ed. G.R. Gibson and R.A. Rastall. West Sussex: Wiley.

Crittenden, R.G., and M.J. Playne. 1996. Production, properties and applications of food-grade oligosaccharides. *Trends in Food Science and Technology* 7: 353–361.

Crittenden, R.G., S. Karppinen, S. Ojanen, et al. 2002. In vitro fermentation of cereal dietary fiber carbohydrates by probiotic and intestinal bacteria. *Journal of the Science of Food and Agriculture* 82: 1–9.

Cummings, J.H., G.T. Macfarlane, and H.N. Englyst. 2001. Prebiotic digestion and fermentation. *American Journal of Clinical Nutrition* 73: 415S–420S.

De Leenheer, L. 1996. Production and use of inulin: Industrial reality with a promising future. In *Carbohydrates as organic raw materials*, vol. III, ed. H. Van Bekkun, H. Röper, and H. Vorgen. Weinheim: Wiley-VCH.

Debruyn, A., A.P. Alvarez, P. Sandra, et al. 1992. Isolation and identification of O-β-D-fructofuranosyl-(2→1)-O-β-D-fructofuranosyl-(2→1)-D-fructose, a product of the enzymatic hydrolysis of the inulin from *Cichorium intybus*. *Carbohydrate Research* 235: 303–308.

Dongowski, G., G. Jacobasch, and D. Schmiedl. 2005. Structural stability and prebiotic properties of resistant starch type 3 increase bile acid turnover and lower secondary bile acid formation. *Journal of Agricultural and Food Chemistry* 53: 9257–9267.

Fava, F., H. Mäkivuokko, H. Siljander-Rasi, et al. 2007. Effect of polydextrose and intestinal microbes and immune functions in pigs. *British Journal of Nutrition* 98: 123–133.

Figdor, S.K., and H.H. Rennhard. 1981. Caloric utilization and disposition of [^{14}C] polydextrose in the rat. *Journal of Agricultural and Food Chemistry* 29: 1181–1189.

Fincher, G.B., and B.A. Stone. 1986. Cell walls and their components in cereal grain technology. *Advances in Cereal Science and Technology* 6: 207–295.

Fleming, S.E., D. Marthinsen, and H. Kuhnlein. 1983. Colonic function and fermentation in men consuming high fiber diets. *Journal of Nutrition* 113: 2535–2544.

Food and Drug Administration. 1993. Food labeling: Mandatory status of nutrition labeling and nutrient content revision, format for nutrition label. *Federal Register* 58: 2079.

Gibson, G.R., and F. Angus. 2000. Leatherhead Ingredients Handbook: Prebiotics and Probiotics. Leatherhead, UK: Leatherhead Food Research Association.

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

Gibson, G.R., H.M. Probert, J. Van Loo, R.A. Rastall, and M.B. Roberfroid. 2004. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews* 17: 259–275.

Grizard, D., and C. Barthomeuf. 1999. Non-digestible oligosaccharides used as prebiotic agents: Mode of production and beneficial effects on animal and human health. *Reproduction Nutrition Development* 39: 563–588.

Guigoz, Y., F. Rochat, and G. Prerruisseau-Carrier. 2002. Effects of oligosaccharide on the faecal flora and non-specific immune system in elderly people. *Nutrition Research* 22: 13–25.

Hara, H., S. Li, M. Sasaki, et al. 1994. Effective dose of lactosucrose on fecal flora and fecal metabolites of humans. *Bifidobacteria Microflora* 13: 51–63.

Harmsen, H.J.M., P. Elfferich, F. Schut, et al. 1999. A 16S rRNA-targeted probe for detection of lactobacilli and enterocci in faecal samples by fluorescent in situ hybridization. *Microbial Ecology in Health and Disease* 11: 3–12.

Hernot, D.C., T.W. Boileau, L.L. Bauer, et al. 2008. In vitro digestion characteristics of unprocessed and processed whole grains and their components. *Journal of Agricultural and Food Chemistry* 56: 10721–10726.

Hopkins, M., J. Cummings, and G. MacFarlane. 1998. Inter-species differences in maximum specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and other simple carbohydrate sources. *Journal of Applied Microbiology* 85: 381–386.

Ito, M., Y. Deguchi, A. Miyamori, et al. 1990. Effect of administration of galacto-oligosaccharides on the human faecal flora, stool weight and abdominal sensation. *Microbial Ecology in Health and Disease* 3: 285–292.

IUP-IUPAC. 1982. Joint commission on biochemical nomenclature and (JCBN): Abbreviated terminology of oligosaccharide chains: Recommendations 1980. *Journal of Biological Chemistry* 257: 3347–3351.

Jabocasch, G., G. Dongowski, D. Schmiedl, et al. 2006. Hydrothermal treatment of Novelose 330 results in high yield of resistant starch type 3 with beneficial prebiotic properties and decreased secondary bile acid formation in rats. *British Journal of Nutrition* 95: 1063–1074.

Jenkins, D.J.A., C.W.C. Kendall, and V. Vuksan. 1999. Inulin, oligofructose and intestinal function. *Journal of Nutrition* 129: 1431S–1433S.

Jie, Z., B. Lou, M. Xiang, et al. 2000. Studies on the effects of polydextrose on physiological function in Chinese people. *American Journal of Clinical Nutrition* 72: 1503–1509.

Juśkiewicz, J., and Z. Zduńczyk. 2002. Lactulose-induced diarrhoea in rats: Effects on caecal development and activities of microbial enzymes. *Comparative Biochemistry and Physiology* 133: 411–417.

Karppinen, S., K. Liukkonen, A.M. Aura, et al. 2000. In vitro fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. *Journal of the Science of Food and Agriculture* 80: 1469–1476.

Kaur, N., and A. Gupta. 2002. Applications of inulin and oligofructose in health and nutrition. *Journal of Biosciences* 27: 703–714.

Kawase, M., A. Pilgrim, T. Araki, et al. 2001. Lactosucrose production using a simulated moving bed reactor. *Chemical Engineering Science* 56: 453–458.

Kelly-Quagliana, K.A., P.D. Nelson, and R.K. Buddington. 2003. Dietary oligofructose and inulin modulate immune functions in mice. *Nutrition Research* 23: 257–267.

Kleessen, B., G. Stoof, J. Proll, et al. 1997. Feeding resistant starch affects fecal and cecal microflora and short chain fatty acids in rats. *Journal of Animal Science* 75: 2453–2462.

Kumemura, M. 1992. Effects of administration of 4G-β-D-galactosylsucrose on fecal microflora putrefactive compounds, short chain fatty acids, weight, moisture, and subjective sensation of defecation in the elderly constipation. *Journal of Clinical Biochemistry and Nutrition* 13: 199–210.

L'Hocine, L., Z. Wang, G. Jian, and S.Y. Xu. 2000. Purification and partial characterization of fructosyltransferase and invertase from *Aspergillus niger* AS0023. *Journal of Biotechnology* 81: 73–84.

Li, B.W. 2010. Analysis of dietary fiber and nondigestible carbohydrates. In *Handbook of prebiotics and probiotics ingredients: Health benefits and food applications*, ed. S.S. Cho and E.T. Finocchiara. Boca Raton, FL: CRC Press.

Manning, T.S., and G.R. Gibson. 2004. Prebiotics. *Best Practice and Research Clinical Gasteroenterology* 108: 975–982.

Marteau, P. 2001. Prebiotics and probiotics for gastrointestinal health. *Clinical Nutrition* 40: 41–45.

Moro, G., I. Minoli, M. Mosca, et al. 2002. Dosage-related bifidogenic effects of galacto- and fructoligosaccharides in formula-fed term infants. *Journal of Pediatrics Gastroenterology and Nutrition* 34: 291–295.

Murphy, O. 2001. Non-polyol low-digestible carbohydrates: Food application and functional benefits. *British Journal of Nutrition* 85: S47–S53.

Mussatto, S.I., and I.M. Mancilha. 2007. Non-digestible oligosaccharides: A review. *Carbohydrate Polymers* 68: 587–597.

Nilsson, K.G.I. 1988. Enzymatic synthesis of oligosaccharides. *Trends in Biotechnology* 6: 256–264.

Ohkusa, T., Y. Ozaki, C. Sato, et al. 1995. Long-term ingestion of lactosucrose increases *Bifidobacterium* spp. in human fecal flora. *Digestion* 56: 415–420.

Okazaki, M., S. Fujikawa, and N. Mutumoto. 1990. Effect of xylooligosaccharide on the growth of bifidobacteria. *Bifidobacteria Microflora* 9: 77–86.

Park, Y.K., and M.M. Almeida. 1991. Production of fructooligosaccharides from sucrose by tranfructosylase from *Aspergillus niger*. *World Journal of Microbiology and Biotechnology* 7: 331–334.

Pederson, A., B. Sanstrom, and J.M.M. van Amelsvoort. 1997. The effect of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. *British Journal of Nutrition* 78: 215–222.

Playne, M.J. 1994. Production of carbohydrate-based functional foods using enzyme and fermentation technologies. *Int Chem Eng Symp Ser* 137: 147–156.

Playne, M.J., and R.G. Crittenden. 2004. Prebiotics from lactose, sucrose, starch, and plant polysaccharides. In *Bioprocesses and biotechnology for functional foods and nutraceuticals*, ed. J.-R. Neeser and J.B. German. New York: Marcel Dekker.

Prenosil, J.E., E. Stucker, and J.R. Bourne. 1987. Formation of oligosaccharides during enzymatic lactose. Part 1: State of art. *Biotechnology and Bioengineering* 30: 1019–1025.

Rastall, R.A., and V. Maitin. 2002. Prebiotics and synbiotics: Towards the next generation. *Current Opinion Biotechnology* 13: 490–496.

Roberfroid, M.B., and J. Slavin. 2000. Nondigestible oligosaccharides.Critical Review in Food Science and Nutrition 40: 461–480.

Roberfroid, M.B., and N. Delzenne. 1998. Dietary fructans. Annual Review Nutrition 18: 117–143.

Roberfroid, M.B. 2007. Prebiotics: The concept revisited. *Journal of Nutrition* 137: 830S–837S.

Roberfroid, M.B. 2008. Prebiotics: Concepts, definition, criteria, methodologies, and products. In *Handbook of prebiotics*, ed. G.R. Gibson and M.B. Roberfroid. Boca Raton: CRC Press.

Roberfroid, M.B., and N.M. Delzenne. 1998. Dietary fructans. *Annual Review of Nutrition* 18: 117–143.

Roberfroid, M.B., J.A.E. Van Loo, and G.R. Gibson. 1998. The bifidogenic nature of chicory inulin and its hydrolysis products. *Journal of Nutrition* 128: 11–19.

Sako, T., K. Mastumoto, and R. Tanaka. 1999. Recent progress on research and applications of non-digestible galacto-oligosaccharides. *International Dairy Journal* 9: 69–80.

Silvi, S., C.J. Rumney, A. Cresci, et al. 1999. Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with feces from Italian and UK donors. *Journal of Applied Microbiology* 86: 521–530.

Stowell, J.D. 2009. Polydextrose. In *Fiber ingredients: Food applications and health benefits*, ed. S.S. Cho and P. Samuel. Boca Raton: CRC Press.

Stowell, J.D. 2010. Prebiotic potential of polydextrose. In *Prebiotics and probiotics science and technology*, ed. D. Charalampopoulos and R.A. Rastall. New York: Springer.

Swennen, K., C.M. Courtin, and J.A. Delcour. 2006. Non-digestible oligosaccharides with prebiotic properties. *Critical Reviews in Food Science and Nutrition* 46: 459–471.

Tanaka, R., H. Takayama, M. Morotomi, et al. 1983. Effects of administration of TOS and *Bifidobacterium breve* 4006 on the human fecal flora. *Bifidobacteria Microflora* 2: 17–24.

Terada, A., H. Hara, S. Kato, et al. 1993. Effect of lactosucrose (4G-β-D-galactosylsucrose) on fecal flora and fecal putrefactive products of cats. *The Journal of Veterinary Medical Science* 55: 291–295.

Tiihonen, K., T. Suomalainen, S. Tynkknen, et al. 2008. Effect of prebiotic supplementation on a probiotic bacterial mixture: Comparison between a rat model and clinical trials. *British Journal of Nutrition* 99: 826–831.

Tuohy, K.M., C.J. Ziemer, A. Klinder, et al. 2002. A human volunteer study to determine the prebiotic effects of lactulose powder on human colonic microbiota. *Microbial Ecology in Health and Disease* 14: 165–173.

Tzortis, G., and J. Vulevic. 2009. Galacto-oligosaccharide prebiotics. In *Prebiotics and probiotics science and technology*, ed. D. Charalampopoulos and R.A. Rastall. New York: Springer.

Van Loo, J., P. Coussement, L. De Leenheer, et al. 1995. On the presence of inulin and oligofructose as natural ingredients in the Western diet. *Critical Reviews in Food Science and Nutrition* 35: 525–552.

Vázquez, M.J., J.L. Alonso, H. Domínguez, et al. 2000. Xylooligo-saccharides: Manufacture and applications. *Trends in Food Science and Technology* 11: 387–393.

Wang, X., I.L. Brown, D. Khaled, et al. 2002. Manipulation of colonic bacteria and volatile fatty acid production by dietary high amylose maize (amylomaize) starch granules. *Journal of Applied Microbiology* 93: 390–397.

Yoshhikawa, J., S. Amachi, H. Shinoyama, et al. 2008. Production of fructooligosaccharides by crude enzyme preparations of β-fructofuranosidase from *Aureobasidium pullulans*. *Biotechnology Letters* 30: 535–539.

Yun, J.W. 1996. Fructooligosaccharides: Occurrence, preparation, and application. *Enzyme and Microbial Technology* 19: 107–117.

Chapter 3
Microbial Species Characteristics and Selection

Lacey M. Guillen

Abstract A systematic screening approach is vital for the selection of successful direct-fed microbials. Only well thought out in vitro testing and selection followed by comprehensive in vivo testing can help ensure a direct-fed microbial product that is maximally beneficial to the host animal. The microorganism must be able to survive transit through the digestive process of the host animal. Survival through the gastrointestinal tract is reliant on acid and bile tolerance of the direct-fed microbial strain. It is also well agreed upon that subsisting in the intestinal tract by colonization for some length of time and the ability to adhere to epithelial cells and/or intestinal mucus is important. It may be advantageous to select the candidate direct-fed microbial strains from the species for which they are intended to benefit. However, in vitro and in vivo testing may show that some strains can colonize and provide benefits across the specific animal species tested. The preparation of direct-fed microbial cultures may affect their in vivo activity, such as the need to activate inducible starch-hydrolyzing enzymes for a product to aid in starch utilization. If multiple strains of microorganisms are to be used, their compatibility and combined effects need to be thoroughly tested.

3.1   Introduction

There is a long history of the use of probiotics for the health benefits of humans, and there is now an ever-growing interest for the successful use of direct-fed microbials in animals. More often than not, live microorganism feed supplements for animals

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are referred to as direct-fed microbials but sometimes are still called probiotics. The U.S. Food and Drug Administration (FDA) uses the term "direct-fed microbial products" to define these products for animals (U.S. FDA 1995). This chapter focuses on the selection criteria for successful direct-fed microbials and the species involved, as listed by the Association of American Feed Control Officials and reviewed and found to be safe by the U.S. FDA and the Center for Veterinary Medicine.

3.2 General Selection Criteria

3.2.1 Acid and Bile Tolerance

Bacteria that are selected as direct-fed microbials or probiotics need to have some degree of acid and bile tolerance to survive the gastrointestinal tract (Chou and Weimer 1999; Tuomola et al. 2001). The digestive process involves an acidic environment, and it is crucial that a direct-fed microbial be able to withstand it (Conway et al. 1987; Brashears et al. 2003). The bile present in the small intestine can disrupt cell membranes of bacteria, resulting in cell death (Gilliland et al. 1984). A study conducted by Gilliland et al. (1984) investigated the ability to establish intestinal growth in dairy calves by two strains of *Lactobacillus acidophilus* with differing levels of bile tolerance. The strain of *L. acidophilus* with the highest degree of bile tolerance had the most growth in the upper small intestines of calves. An increase in the colonization of direct-fed microbials in the upper intestine might be important for controlling the proliferation of intestinal pathogens as they enter the intestinal tract (Gilliland et al. 1984).

3.2.2 Adherence to Epithelial Cells and Intestinal Mucus

Screening potential direct-fed microbial strains for the ability to adhere to epithelial cells and mucosal surfaces of the host species is an important selection criterion for successful colonization (Rojas et al. 2002; Conway et al. 1987; Salminen et al. 1996). Rojas et al. (2002) extracted, purified, and characterized a cell surface protein from *L. fermentum* strain 104R. This surface protein demonstrated an affinity to bind to small intestinal mucus from 35-day-old piglets and pig gastric mucin.

The use of in vitro assays as a screening tool for selecting direct-fed microbials needs to be validated to eliminate some of the variables seen across similar experiments (Blum et al. 1999). Bacterial growth conditions, such as types of growth media used and incubation times and temperatures, can affect the adhesion of direct-fed microbial and probiotic cultures. A more standardized approach to these assays can increase their reliability.

Adhesion testing for the proposed colonization site of the direct-fed microbial may also be important. The differences between the mucosal surfaces of the small and large intestine may influence colonization by microorganisms. Therefore, an adhesion model would ideally combine the three components of the mucosa – epithelial cells, mucus covering epithelial cells, mucosa-associated microbiota – although it would be a difficult undertaking (Ouwehand and Salminen 2003).

3.2.3 Host Specificity

The concept of host specificity is a debated topic. Some early probiotic studies support host specificity (Gilliland et al. 1975; Barrow et al. 1980), whereas some current studies do not (Rinkinen et al. 2003). The in vitro studies conducted by Rinkinen et al. (2003) showed that the lactic acid bacterial strains they were testing adhered to human, canine, possum, bird, and fish mucus. Their group argued that it is not the host specificity that drives the adhesion properties but the individual strain's adhesion ability. Other studies have produced similar findings, such as the ability of human-derived probiotic strains to adhere to fish mucus (Nikoskelainen et al. 2001). This may be true in some instances, but it must be remembered that these are in vitro assays and it is difficult to simulate the conditions of adhesion in vivo (Ouwehand and Salminen 2003). When using an in vitro test such as this for screening purposes, it is necessary to correlate the results with follow-up in vivo testing.

3.3 Strain Specificity and Direct-Fed Microbial Preparation

3.3.1 Strain Specificity

The activities of a successful probiotic or direct-fed microbial are strain-specific. All species of direct-fed microbials do not behave the same way, and neither do different strain types within a given species (Gilliland 2001). Brashears et al. (2003) tested 686 bacterial isolates from cattle manure regarding their ability to inhibit a four-strain mixture of *Escherichia coli* O157:H7. This was the first round of screening for a potential competitive exclusion direct-fed microbial to reduce *E. coli* O157:H7 in cattle. The next step of isolate and strain selection was to test the 75 isolates demonstrating the greatest bactericidal effects for acid and bile tolerance. The strains carried on from this screening step were subjected to further *E. coli* O157:H7 inhibition analyses in manure and rumen fluid as well as tests for antibiotic resistance. In the end, this step-by-step process identified a strain that was the best candidate for this competitive exclusion direct-fed microbial. The next step in the process would be to test the isolate for its activity in cattle-feeding trials.

3.3.2   Preparing Cultures to Promote Desired Benefits

The direct-fed microbial must be selected based on its ability to impart the desired benefit to the host. In addition, the strain selected must be produced for use in a manner that promotes the action for which it is chosen. An example is the selection of a direct-fed microbial to aid in starch utilization of the host animal. The direct-fed microbial would need to be produced under conditions to induce the production of extracellular starch-degrading enzymes. Ryan et al. (2006) screened 42 bifidobacterial strains for their ability to degrade starch, amylopectin, and pullulan. All of the cultures were tested by growing them in a carbon- and energy-limiting basal medium with added 1% starch, amylopectin, or pullulan. They found that 19 of the 42 strains could utilize starch, but only 11 could utilize amylopectin and pullulan. The researchers also determined that the bacterial enzymes were extracellular. These results support both strain specificity and the need to prepare the culture to be used in a manner that promotes the desired activity.

3.3.3   Preparing Cultures for Environmental Stressors

Direct-fed microbial cultures may also be selected and prepared based on the environmental stressors they are likely to encounter in an animal or food system. Interestingly, Jan et al. (2001) found that *Propionibacterium freudenreichii* strain SI41 underwent both genotypic and phenotypic changes owing to acid stress adaptation. Triggering an acid-adaptive response might aid in the survival of larger numbers of direct-fed microbials in a host animal.

3.3.4   Preparing Multiple Strain Direct-Fed Microbials

In some cases, a mixture of experimentally selected strains for direct-fed microbials may be necessary to deliver the intended benefit to the host animal (Gilliland 2001). If more than one strain is used, they must be tested for their compatibility with one another in vitro and in vivo. It may be necessary to grow and prepare each culture separately to ensure maximal growth for each strain.

An example of a multiple strain product is the use of selected *Propionibacterium freudenreichii* strains in combination with *L. acidophilus* strains for cattle-feeding trials. This combination of lactate-producing and lactate-utilizing direct-fed microbials has been well characterized through a variety of cattle-feeding trials to refine the optimal strain combinations and doses in vivo. The cultures are administered as a rehydrated freeze–dried preparation that is then mixed into the feed each day (Elam et al. 2003; Younts-Dahl et al. 2005; Vasconcelos et al. 2008).

3.3.5   Stability of Direct-Fed Microbials in Feed Products

The stability of the culture for commercial production needs to be addressed. The direct-fed microbial product must retain its desired properties and remain viable during production, distribution, and storage of the feed product (Gilliland 2001). The appropriate time to add the culture to the product can be determined through experimentation and shelf-life studies. Some products currently on the market are added to the feed at the time of mixing as a lyophilized preparation. The lyophilized product is stored frozen until the time of use. Other technologies exist as well, such as microencapsulation, which might be useful for keeping a culture viable throughout the feed-making process.

3.4   Species Involved

The following lists of species of direct-fed microbials are derived from the Association of American Feed Control Officials 1999 official publication (AAFCO 1999). The lists represented in this chapter are broken down by genus and microorganism type.

Lactobacillus

- *L. acidophilus*
- *L. reuteri*
- *L. casei*
- *L. fermentum*
- *L. plantarum*
- *L. brevis*
- *L. buchneri* (cattle only)
- *L. delbrueckii*
- *L. helveticus*
- *L. lactis*
- *L. bulgaricus*
- *L. cellobiosis*
- *L. curvatus*
- *L. farciminis* (swine only)

Bifidobacterium

- *B. adolescentis*
- *B. animalis*
- *B. bifidum*
- *B. infantis*
- *B. longum*
- *B. thermophilum*

Blum, S., R. Reniero, E.J. Schiffrin, R. Crittenden, T. Mattila-Sandholm, A.C. Ouwehand, and S. Salmin. 1999. Adhesion studies for probiotics: Need for validation and refinement. *Trends in Food Science and Technology* 10: 405–410.

Brashears, M.M., D. Jaroni, and J. Trimble. 2003. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherchia coli* O157:H7 in cattle. *Journal of Food Protection* 66: 355–363.

Chou, L., and B. Weimer. 1999. Isolation and characterization of acid- and bile-tolerant isolates from strains of *Lactobacillus acidophilus*. *Journal of Dairy Science* 82: 23–31.

Conway, P.L., S.L. Gorbach, and B.R. Goldin. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *Journal of Dairy Science* 70: 1–12.

Elam, N.A., J.F. Gleghorn, J.D. Rivera, M.L. Galyean, P.J. Defoor, M.M. Brashears, and S.M. Younts-Dahl. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Proionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain 0157 shedding on finishing beef steers. *Journal of Animal Science* 81: 2686–2698.

Gilliland, S.E. 2001. Probiotics and prebiotics. In *Applied dairy microbiology*, 2nd ed, ed. E.H. Marth and J.L. Steele. New York: Marcel Dekker.

Gilliland, S.E., M.L. Speck, and C.G. Morgan. 1975. Detection of *Lactobacillus acidophilus* in feces of humans, pigs, and chickens. *Applied Microbiology* 30(4): 541–545.

Gilliland, S.E., T.E. Stanley, and L.J. Bush. 1984. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. *Journal of Dairy Science* 67: 3045–3051.

Jan, G., P. Leverrier, V. Pichereau, and P. Boyaval. 2001. Changes in protein synthesis and morphology during acid adaptation of *Propionibacterium freudenreichii*. *Applied and Environmental Microbiology* 67: 2029–2036.

Nikoskelainen, S., S. Salminen, G. Bylund, and A.C. Ouwehand. 2001. Characterization of the properties of human- and dairy-derived probiotics for prevention of infectious diseases in fish. *Applied and Environmental Microbiology* 67: 2430–2435.

Ouwehand, A.C., and S. Salminen. 2003. In vitro adhesion assays for probiotics and their in vivo relevance: A review. *Microbial Ecology in Health Disease* 15: 175–184.

Rinkinen, M., E. Westermarck, S. Salminen, and A.C. Ouwehand. 2003. Absence of host specificity for in vitro adhesion of probiotic lactic acid bacteria to intestinal mucus. *Veterinary Microbiology* 97: 55–61.

Rojas, M., F. Ascencio, and P.L. Conway. 2002. Purification and characterization of a surface protein from *Lactobacillus fermentum* 104R that binds to porcine small intestinal mucus and gastric mucin. *Applied and Environmental Microbiology* 68: 2330–2336.

Ryan, S.M., G.F. Fitzgerald, and D. Sinderen. 2006. Screening for and identification of starch-, amylopectin-, and pullulan-degrading activities in bifidobacterial strains. *Applied and Environmental Microbiology* 72: 5289–5296.

Salminen, S., E. Isolauri, and E. Salminen. 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. *Antonie Van Leeuwenhoek* 70: 347–358.

Tuomola, E., R. Crittenden, M. Playne, E. Isolauri, and S. Salminen. 2001. Quality assurance criteria for probiotic bacteria. *American Journal of Clinical Nutrition* 73(Suppl): 393S–398S.

Uas 1995. "Direct-fed microbial products. Section 689.100." http://www.fda.gov/ICECI/Compliance Manuals/CompliancePolicyGuidanceManual/ucm074707.html. Accessed May 14, 2010.

Vasconcelos, J.T., N.A. Elam, M.M. Brashears, and M.L. Galyean. 2008. Effects of increasing dose of live cultures of *Lactobacillus acidophilus* (strain NP 51) combined with a single dose of *Propionibacterium freudenreichii* (strain NP 24) on performance and carcass characteristics of finishing beef steers. *Journal of Animal Science* 86: 756–762.

Younts-Dahl, S.M., G.D. Osborn, M.L. Galyean, J.D. Rivera, G.H. Loneragan, and M.M. Brashears. 2005. Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. *Journal of Food Protection* 68: 6–10.

Chapter 4
Genomics of Probiotic–Host Interactions

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Abstract Modern molecular methods are currently growing exponentially, enhancing our ability to evaluate the microbiome of animals and the interaction of the complex consortium of organisms with the host. Current limitations include a lack of ability to propagate many types of bacteria that might prove to be valuable direct-fed microbial resources. Also lacking are genomic tools for animal origin probiotics. During the past few years, however, an incredible revolution in the area of evaluating the microbiome of animals has been initiated with the advent of molecular methods such as the pyrosequencing-based bTEFAP. Metagenomic molecular and bioinformatics tools and resources are likely to continue to revolutionize our understanding of how probiotics interact and enhance gut health in animals, particularly those of economic importance. Future directions in whole-community transplants (e.g., fecal bacterial transplant therapy) seem a logical step from single bacterial direct-fed microbials especially as modern methods for ensuring and monitoring safety and efficacy are now becoming available.

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4.1   Introduction

The typical gastrointestinal tract of the mammals and birds contains several hundreds of diverse, and complex species of nonpathogenic bacteria, collectively known as the gut microbiota. A healthy newborn acquires the microbiota during the first few weeks of life. Upon colonization, the microbiota establishes a symbiotic relationship with the host, which remains relatively unchanged throughout life. It plays a pivotal role in the host's metabolism, gut integrity and motility, intestinal immune response, and protection against pathogenic bacteria. Direct-fed microbials, traditionally known as probiotics (or beneficial bacteria), are derived from the normal gut microbiota and are widely used as feed additives to augment health and growth and to control as well as treat bacterial infections in livestock and poultry. The joint United Nations Food and Agricultural Organization/World Health Organization (FAO/WHO) group report defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." There are several lines of evidence that support the beneficial effects of veterinary probiotics. Yet, the mechanisms by which they operate inside the animal gut are poorly understood at the molecular level. In 1995, for the first time, a genome of bacteria, namely *Haemophilus influenzae*, was completely sequenced, revolutionizing the field of microbial genomics. Since then, whole-genome sequencing of a large number of bacteria including, for example, probiotic bacteria such as *Bifidobacterium longum* NCC2705 has been completed. Importantly, genome sequences of probiotic bacteria provide information on the evolution of the gut microbiota, genome diversity, functional properties, and probiotic–host interactions. In this chapter, we highlight some of the current knowledge on genome technologies, genomics of probiotics, and genomics of probiotic–animal interactions.

4.2   Genomic Tools to Study Gut Bacteria

A wide range of techniques are currently available to study the gut microbiota in animals and poultry. Much of the current knowledge on the characteristics of the microbiota has originated from studies based on the conventional culturing technique. This technique is effective in the evaluation of the viability, cultivability, metabolic activity, infectivity, and antibiotic susceptibility of the gut bacteria. Furthermore, typing of individual isolates can be useful for a better understanding of the epidemiological and ecological aspects of the gut microbiota. Unfortunately, this approach has several intrinsic limitations. First, a large number of bacteria present in the gut cannot be cultured even today, primarily because of a lack of knowledge of their optimal, or even their minimal, growth requirements. With the present laboratory conditions and even with heroic efforts (hundreds of media and environmental combinations), only about 20% of the gut bacteria can be grown. Thus, it is not surprising that the total viable counts are typically lower than direct microscopic

counts of the gut bacteria using fluorescent dyes. Second, the existence of microbe–microbe and microbe–host interactions in the gut is an impediment to successful isolation of the bacteria. Third, the outcome of bacterial culture depends on appropriate handling, storage, transportation, and processing of samples. Finally, currently available phenotypic and biochemical tests have limited utility for proper identification and classification of the isolates.

A variety of molecular biology-based techniques are increasingly being used by many researchers to study the diversity of gut microbiota (Bailey et al. 2010; Callaway et al. 2010; Callaway et al. 2009; Dethlefsen et al. 2006; Dowd et al. 2008a, c; Ritchie et al. 2008; Suchodolski et al. 2008). These techniques involve extraction of either DNA or RNA from gut samples, following which a conserved gene present in the bacterial genome – e.g., 16S ribosomal RNA (rRNA) gene – is amplified using universal primers in a standard polymerase chain reaction (PCR) (Zoetendal et al. 2004). The 16S rRNA gene (~1,550 bp) is by far the most frequently used gene in these techniques because not only is it universally present in bacteria, it is highly conserved. Furthermore, the availability of thousands of 16S rRNA gene sequences in GenBank makes it an attractive candidate gene for comparative genomic studies. Other less commonly targeted genes include the 16S–23S internal transcribed spacer (ITS) region and the chaperonin (cpn60) sequences (Desai et al. 2009). Following amplification, the PCR products are separated using gel electrophoresis to generate a "fingerprint" of the gut bacterial community unique to individual animals. This approach allows concurrent analysis and comparison of bacterial communities in multiple gut samples. Examples of routinely used techniques include denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism (T-RFLP). The separation of amplified sequences in the DGGE (Dowd et al. 2008b) and TGGE systems is based on a linear gradient of denaturing agents and a gradient of temperature, respectively. Both DGGE and TGGE are economical and easy to use. The major limitation of these techniques is that they generate small, poorly resolving bands on the gel.

In addition to generating a profile, the bacterial communities can be quantified using T-RFLP. With this method, amplification of the target gene is achieved with a fluorescence-labeled primer; the amplicons are then digested with a restriction enzyme. The digested products are further separated by electrophoresis, and fluorescent signals from the amplicons are detected and quantified, creating a profile of that sample. T-RFLP is highly reproducible, but the bands cannot be extracted for further sequencing. An alternative to generating patterns is to clone the amplified products, sequence them, and compare them with sequences available in databases (Forney et al. 2004; Zoetendal et al. 2004). Novel, high-throughput sequencing platforms such as 454-pyrosequencing and the bTEFAP methodology are increasingly being used in the field of gut microbiology. A major advantage of these techniques is that cloning of PCR products for sequencing is circumvented. Using this platform, several thousand sequences from the gut microbiota can be generated and analyzed within a few hours (Bailey et al. 2010; Callaway et al. 2009, 2010;

Dethlefsen et al. 2008; Dowd et al. 2008a, c; Suchodolski et al. 2009). Various molecular techniques have been employed to quantify gut bacterial communities, including quantitative real-time PCR assays (Desai et al. 2009; Lubbs et al. 2009), fluorescent in situ hybridization (FISH) (Janeczko et al. 2008), RNA dot blot hybridization (Lipski et al. 2001; Sghir et al. 2000), and flow cytometry. Among these techniques, FISH is most commonly used as it also provides information about the specific location of the bacteria in the gut epithelium.

Metagenomics and transcriptomics are emerging as powerful approaches for gaining valuable information on the biology of uncultivable gut bacteria. With metagenomics, DNA that has been isolated from a gut sample is cloned into a suitable vector to create metagenomic libraries, which are then screened for specific markers or phenotypic traits or are randomly sequenced. This approach generates a staggering amount of information about the gene pool and the functional potential of the microbiome. With transcriptomics, mRNA is analyzed from a gut sample, which yields information on the gene expression pattern. These novel techniques provide us with an unprecedented opportunity to understand the microbial–host interactions in health and disease.

4.3 Probiogenomics

Bacterial species belonging to the genus *Lactobacillus* and *Bifidobacterium* are the most commonly utilized veterinary probiotic supplements. Examples of *Lactobacillus* species that have been approved for veterinary use in the United States include *acidophilus*, *brevis*, *bucheri*, *casei*, *cellobiosus*, *curvatus*, *lactis*, *plantarum*, and *reuteri*. The most commonly used *Bifidobacterium* species include *adolescentis*, *animalis*, *bifidum*, *infantis*, *lactis*, *longum*, and *thermophilum*. During the current decade, determination and analysis of complete genome sequences of members of the genera *Lactobacillus* and *Bifidobacterium* have received considerable attention as they offer new insights into the genetics, physiology, ecology, and molecular basis of interactions of probiotics with host and other gut bacteria. The genome information can also be used for further development of strains and assessment of the safety of probiotics. The evolution and genetic diversity can be better understood by comparing the genomes of various probiotic species. Recently, the term "probiogenomics" has been proposed to denote the sequencing and analysis of probiotic genomes.

To date, nine *Bifidobacterium* strains have been completely sequenced, and the full genome sequencing of at least 12 more strains are currently underway in many laboratories. Most, if not all, of these strains are originally isolated from human feces or gut. Complete genome sequencing of animal-origin bifidobacteria has not yet been reported in the literature. Table 4.1 summarizes the genome characteristics of select bifidobacteria strains. Not surprisingly, these organisms share many genes responsible for their adaptation to the gut environment. For instance, once in the gut, they encounter harsh conditions such as bile acids. To survive this condition,

Table 4.1 Genome features of *Lactobacillus* and *Bifidobacterium* species isolated from mammals and birds

Probiotic	Strain	Origin	Genome (Mbp)	Genes	Structural RNAs	Pseudo genes	GenBank	Ref
Bifidobacterium longum	NCC2705	Human feces	2.2	1,798	70	None	AE014295	Schell et al. (2002)
Bifidobacterium longum	DJO10A	Human feces	2.3	2,061	73	None	CP000605	Lee et al. (2008)
Bifidobacterium longum subsp. *infantis*	ATCC 15697	Human gut	2.8	2,588	94	94	CP001095	Sela et al. (2008)
Bifidobacterium animalis subsp. *lactis*	AD011	Human feces	1.9	1,527	59	17	CP001213	Kim et al. (2009)
Bifidobacterium animalis subsp. *lactis*	Bl 04	Human feces	1.9	1,631	64	None	CP001515	Barrangou et al. (2009)
Lactobacillus johnsonii	FI9785	Poultry gut	1.7	1,780	70	None	FN298497	Wegmann et al. (2009)
Lactobacillus crispatus	ST1	Poultry crop	2.0	2,100	76	None	FN692037	Ojala et al. (2010)
Lactobacillus plantarum	WCFS1	Human mouth	3.3	3,135	86	42	AL935263	Kleerebezem et al. (2003)
Lactobacillus salivarius	UCC118	Human gut	1.8	1,864	99	49	CP000233	Claesson et al. (2006)
Lactobacillus rhamnosus	GG	Human gut	3.0	2,985	72	None	FM179322	Kankainen et al. (2009)
Lactobacillus reuteri	JCM 1112	Human gut	2.0	1,901	81	None	AP007281	Morita et al. (2008)
Lactobacillus gasseri	ATCC 33323	Human gut	1.8	1,898	98	48	CP000413	Azcarate-Peril et al. (2008)
Lactobacillus acidophilus	NCFM	Human gut	1.9	1,936	74	None	CP000033	Altermann et al. (2005)
Lactobacillus johnsonii	NCC 533	Human feces	1.9	1,918	97	None	AE17198	Pridmore et al. (2004)

many bifidobacteria encode for bile salt hydrolase, which causes the deconjugation of glycine- or taurine-linked bile salts.

On the other hand, probiotics may carry some unique genes that offer an advantage for survival in the gut milieu. Historically, the first probiotic bacterium to be completely sequenced was *B. longum* NCC2705 isolated from human infant feces. Analysis of its genome has revealed a novel gene, *BL065*, which encodes for a fimbriae-like, cell-surface motif that could mediate attachment and colonization of host cells (Schell et al. 2002). It is noteworthy that fimbriae had not been previously identified in any of the *Bifidobacterium* species. Intriguingly, a serine protease inhibitor (serpin) (BL0108) with similarities to eukaryotic serpins was also discovered in this study. Recently, a similar serpin gene with 96% homology to BL0108 has been uncovered in the genome of *B. longum* subsp. *infantis* strain ATCC15697 (Sela et al. 2008). Serpins, never before described in bacteria, are known to modulate protease functions in inflammation, coagulation, fibrinolysis, and phagocytosis (Silverman et al. 2001). This suggests a novel mechanism by which bifidobacteria could modulate the host immune response.

The ability to utilize larger sugar substrates is illustrated by the discovery of the β-fructofuranosidase gene in the genome of *B. animalis* subsp. *lactis* AD011 (Kim et al. 2009). The major function of β-fructofuranosidase is to degrade the bifiodogenic factors such as short-chained fructooligosacharides. In contrast, the host or other bacteria such as *E. coli* cannot breakdown these complex carbohydrates for energy (Ryan et al. 2005). Recently, the genomes of both *B. animalis* subsp. *lactis* strains DSM 10140 and B1-04 have been reported to carry four genes (*cap*A, *cap*B, *ebp*S, *fbp*) that may function as cell surface ligands for the host cell receptors (Barrangou et al. 2009). In contrast, such cell surface proteins have not been found in the genome of *B. longum* subsp. *infantis* strain ATCC15697, but it codes for solute-binding proteins (SBPs) that perhaps mediate interaction with the host (Sela et al. 2008). A unique feature that differentiates the genome of *B. longum* strain DJO10A from other bifidobacteria genomes is the 10.2-kb gene cluster encoding lanthionine-containing antibiotic peptides (lantibiotics) (Lee et al. 2008). Bactericidal activities of lantibiotics offer an advantage for this probiotic to survive in the highly competitive environment in the gut.

Lactobacillus plantarum WCFS1 isolated from human saliva was the first *Lactobacillus* bacterium to be completely sequenced (Kleerebezem et al. 2003). Since then, 20 more *Lactobacillus* strains have been completely sequenced. The genomes of about 40 more strains are currently being sequenced, and the results are expected within the next few years. The general features of a few *Lactobacillus* genomes are presented in Table 4.1. Recently, *L. johnsonii* strain FI9785 isolated from poultry gut has been completely sequenced (Wegmann et al. 2009). The genome has a circular 1,755,993 bp chromosome with a guanine–cyosine (GC) content of 34%. The G+C percent is similar to that of the human strain *L. johnsonii* NCC 533 (34%), but it is the lowest when compared with most, if not all, other human isolates such as *L. plantarum* (44%), and *L. rhamnosus* (46%). It contains about 1,710 genes, four rRNA operons, 53 tRNA genes, and one complete prophage genome. Furthermore, it contains two circular plasmids, p9785S

(3,471 bp) and p9785L (25,652 bp) with GC contents of 36% and 30%, respectively. Recently, the complete genome sequence of *L. crispatus* strain ST1, originally isolated from the crop of a chicken, has been determined (Ojala et al. 2010). It has a 2.04 Mbp, 37% G+C, single circular chromosomal replicon containing 64 tRNA genes, four rRNA operons, and two CRISPR loci. This study also identified 2,024 probable coding regions comprising 77% of the genome. Of these, 10%, and 13% of the conserved coding sequences (CDSs) have been noted as conserved and novel, respectively.

The genome of *L. plantarum* WCFS1 is the largest *Lactobacillus* genome so far sequenced (Kleerebezem et al. 2003). The 3,308,279-bp genome has been predicted to encode for more than 200 extracellular proteins, containing a signal peptide and at least one anchoring domain. It has been suggested that these proteins may function as adhesion proteins for cell receptors in microbe–host interactions. An interesting feature of *L. salivarius* genome is that it contains about four or five copies of a 242-kb megaplasmid pM118, and it contains nonessential genes that play a role in the pentose phosphate pathway, catabolism of rhamnose and sialic acid, and utilization of sorbitol (Claesson et al. 2006). This plasmid has not been identified before in other lactic acid bacteria. Because of this plasmid, *L. salivarius* is able to utilize a carbon source efficiently for energy compared to other bacteria. Analysis of the genome sequence for *L. rhamnosus* ATCC 53103 has revealed three gene clusters encoding surface proteins with a C-terminal WxL domain (Morita et al. 2009). Proteins with this domain are also identified in *L. plantarum* (19 proteins) and *L. sakei* (15 proteins). Recently, these proteins have been shown to interact with the peptidoglycan. Although the exact functions are unknown, these proteins are predicted to play a role in bacteria–bacteria interactions. Following whole-genomic analysis of *L. rhamnosus* GG, a unique gene cluster (*SpaCBA*) encoding for pilus was uncovered (Kankainen et al. 2009). Notably, it has been demonstrated that spaCBA is indispensible for adhesion of the bacteria to human intestinal mucus; thus, it contributes to a prolonged stay of the probiotic in the intestine. In contrast, *L. rhamnosus* strain LC705, which lacks *spaCBA*, cannot persist in the intestine for an extended period (Kankainen et al. 2009). As many as 14 genes encoding for mucus-binding proteins, the highest number for lactobacilli sequenced so far, have been discovered in the genome *L. gasseri* ATCC 33323 (Azcarate-Peril et al. 2008). Based on earlier studies with similar mucus-binding domain-containing proteins, these proteins are predicted to mediate adhesion to mucin secreted by the host cells. The genome also encodes for several exopolysaccharides (EPSs) involved in adhesion of the bacteria to the gut. Genes encoding the cell-wall anchor motif and mucin-binding glycoproteins have been identified in the genome of *L. acidophilus* NCFM (Altermann et al. 2005). The ability of *L. acidophilus* NCFM to bind fibronectin is illustrated by the presence of the fibronectin-binding protein FbpA. Similar analogous proteins are also found in other lactobacilli, such as *L. johnsonii* and *L. plantarum*. Many proteins that promote binding of *L. johnsonii* NCC 533 to the gut are noteworthy. These include three mucus-binding proteins, glycosylated fimbriae, and immunoglobulin A (IgA) protease (Pridmore et al. 2004).

4.4   Genomics of Probiotic–Host Interactions

Molecular knowledge on the nature and consequences of probiotic–host interactions is invaluable for determining their use in animals. Little research has yet focused on the interaction between veterinary species and probiotics at the molecular level, however. Although the information discussed here comes from studies on probiotics intended for human use, the general principles of interactions are pertinent for animal probiotics as well.

4.4.1   Overview of Probiotic–Host Interactions

Most commonly, probiotics interact with two types of cell in the gut: intestinal epithelial cells and dendritic cells. Specifically, this interaction occurs between the host receptors and probiotic ligands, the molecular details of which are not completely understood. The host receptors, called pattern recognition receptors (PRRs), are recognized by the microorganism-associated molecular patterns (MAMPs). These MAMPs are most commonly located on the bacterial surface, although some are secreted in the gut lumen. It is noteworthy that MAMPs are not exclusive to probiotics but can be expressed by any bacterial species, including pathogenic bacteria. Examples of probiotic MAMPs include flagella, fimbriae, secreted proteins (e.g., p40), SlpA, cell wall-associated polysaccharides (CPSs), lipoteichoic acid, lipopolysaccharide (LPS), and peptidoglycan. Toll-like receptors (TLRs), located on the host cell surface, are the most studied PRRs in mammals. Of the ten well-known TLRs, bacteria are capable of interacting only with TLR1, TLR2, TLR4, TLR5, and TLR9 (Takeda et al. 2003). At present, it is not clear how PRRs on the host cells differentiate probiotic ligands from those of pathogens. It is possible that structure, accessibility, and cellular localization of MAMPs may determine the nature of the interaction. Following activation by MAMPs, the PRRs induce intracellular signaling through the NF-κB and mitogen-activated protein kinase (MAPK) pathways. This in turn activates transcription of genes such as those involved in host defense mechanisms; thus, probiotics induce expression of defensin (Schlee et al. 2007), proinflammatory cytokines such as interleukin-8 (IL-8) (Granato et al. 2004), and tumor necrosis factor α (TNFα) (Matsuguchi et al. 2003). They also induce mucus secretion (Mack et al. 2003) and antiapoptosis (Yan et al. 2007).

In addition to TLRs, the host cell contains the nucleotide-binding oligomerization domain (NOD) family of proteins, NOD-1 and NOD-2, which function as cytosolic pattern recognition receptors. Upon activation by bacterial peptidoglycans, these receptors induce host-signaling pathways similar to TLRs. As stated above, these interactions can be mediated by both pathogenic and probiotic bacteria. Although poorly understood, the final response is influenced by both host and probiotic factors.

4.4.2   Host Side of Probiotic–Host Interactions

The availability of complete bovine, swine, and chicken genomic sequences offers tremendous opportunities to understand the molecular basis of probiotic–host interactions in the gut. Of particular interest is the study of probiotic-induced alterations of host gene expression. At this time, DNA microarray technology is widely employed to study concurrently the differential expression of a large fraction of the genome between a control and the host inoculated with probiotics. Results obtained from microarray analysis are further validated by quantitative real-time reverse transcription (RT)-PCR. Because probiotics carry out a wide range of functions, it is not surprising to learn that they modulate multiple host genes such as those involved in RNA transcription and processing, protein synthesis and transport, metabolism, cell proliferation, cell adhesion, and protein degradation via ubiquitinization. Using microarrays, a limited number of studies have been carried out in humans, mice, and poultry. To our knowledge, however, such detailed study has not been attempted in cattle or swine. The response of the host includes up-regulation and/or down-regulation of genes that are involved in the above-mentioned functions. The results of some of these published studies are summarized in Table 4.2.

A DNA microarray-based study has identified the effects of *Lactobacillus casei* strain Shirota or *Bifidobacterium breve* strain Yakult on gene expression in small intestine epithelial cells or colonic epithelial cells isolated from germ-free BALB/c mice (Shima et al. 2008). When compared with *B. breve* Yakult, *L. casei* Shirota up-regulated more genes in the small intestine; however, both down-regulated a similar number of genes. Many of these up-regulated genes play a role in the defense against pathogens – their growth and development, metabolism, and transport. In contrast, the number of both up- and down-regulated genes in colonic epithelial cells was greater with *B. breve* Yakult than with *L. casei* Shirota. The up-regulated genes are those involved in cell communication, growth and development, and metabolism. It is clearly evident from these findings that the type of probiotic and the regions of the gut determine the type of host gene expression.

Interestingly, the signature of host gene expression depends not only on the type of probiotic strain but also on its components. Indeed, the only microarray analysis on chicken cecal tonsil lymphocytes treated with the peptidoglycan-enriched cell envelope extract (PECE), DNA, and cell envelope fractions of *Lactobacillus acidophilus* revealed up-regulation of 18, 9, and 0 genes, respectively (Brisbin et al. 2008). In this study, DNA was found to be the superior stimulus among the cellular components and enhanced the expression of many genes belonging to the immune system such as β_2-microglobulin, major histocompatibility complex (MHC) class I heavy chain, caspase 3, CD25, CD44, CD45, c-myc, IL-2α receptor (CD25), invariant chain, STAT2, and STAT4.

Most probiotic–host interaction studies are focused on an individual probiotic strain. However, multispecies probiotic supplements are commonly used nowadays. In this scenario, it is essential to understand how hosts are stimulated by these supplements for identifying suitable combination of probiotics. For instance, *Escherichia*

Table 4.2 Probiotic-induced gene expression in host cells

Probiotic	Host	Major up-regulated host genes	Reference
Lactobacillus acidophilus NCFM	Murine dendritic cells	IFN-beta, IL-12, IL-10	Weiss et al. (2010)
Lactobacillus acidophilus NCFM	Caco-2 cells	CCL2, PTX3, TNFRSF9	Wang et al. (2009b)
Lactobacillus rhamnosus GG	C57BL/6 J mice	Dusp3, Areg, CDK2, Ku70, FoxF2, Errb2, Sox4, Jak2, Ho-1	Lin et al. (2008)
Lactobacillus casei Shirota	S.I epithelial cells of BALB/c mice	Cryptdin 1–3, matrilysin, PPAR-γ, intestinal fatty acid binding protein, colipase, phopholipaseA2	Shima et al. (2008)
Escherichia coli Nissle 1917	Polarized intestinal epithelial cells, T84	CLARP, IKB, IL4R, MIP3α, NFATC3, NF-IL6, NFκB p65, PSMD12 p55, TNFAIP3	Zyrek et al. (2007)
Lactobacillus GG	Mouse colonic intestinal epithelial cell line	Hsp70-3, Hsp68, Hsp27 internal deletion variant b, DNA cytosine methyltransferase, Protein tyrosine phosphatase, nonreceptor type 21, nexin 6, Hsp25, heparin-binding EGF-like growth factor precursor	Tao et al. (2006)

coli strain 6-1, isolated from a healthy infant, has been shown to up-regulate and down-regulate 155 and 177 genes, respectively, in Caco-2 cells (Panigrahi et al. 2007). In contrast, *Lactobacillus plantarum* has been found to up-regulate and down-regulate 45 and 36 genes, respectively. However, when incubated together, these bacteria have been shown to up-regulate 27 genes and down-regulate 59 genes. Recently, it has been reported that co-colonization of *B. thetaiotaomicron* and *B. longum* induce maximum expression of interferon (IFN)-responsive genes such IFN-induced protein with TPR2 (Ifit2), IFNα-inducible protein (Isg 15), and IFN-induced protein with TPR1 (Ifit1) in gnotobiotic mouse cecal epithelial cells (Sonnenburg et al. 2006). In contrast, although *B. thetaiotaomicron* alone can up-regulate these genes, the level of expression is low. Collectively, these results indicate the diversity and complexity of the host response to multiple probiotics and that probiotics may not be selected solely based on results with single probiotic bacteria.

Table 4.3 Probiotic-induced changes in the gut microbiota

Probiotic(s)	Dose	Type of animals	Reported probiotic effects	Reference
Propionibacterium P15	10×10^9 cfu/day	Steers	Significantly increased the number of protozoa, particularly *Entodinium* and decreased the number of amylolytic bacteria	Ghorbani et al. (2002)
L. reuteri DSM 16350, *E. facium* DSM 16211, *B. animalis* DSM 16284, *Pediococcus acidilactici* DSM 16210, *L. salivarius* DSM 16351	1×10^{10} cfu/kg of feed	Cobb broilers	Significantly increased the cecal population of *Lactobacillus* and *Bifidobacterium*	Mountzouris et al. (2010)
L. acidophilus, L.casei, B. bifidium, E.faecium	1×10^8 cfu/g of feed	Cobb broilers	Significantly increased the ileal population of *Lactobacillus*	Smirnov et al. (2005)
E. faecium NCIMB 11508 (non-GM) or *E. faecium* NCIMB 11508 containing glucanase (GM)	1×10^5 cfu/g of feed	Chickens	Non-GM and GM significantly increased and decreased the relative amount of *E. faecalis*, respectively	Netherwood et al. (1999)
E.faecium EK13	1×10^9 cfu/ml; 2 ml/piglet	Slovak White piglets	Significantly increased intestinal enterococci populations	Strompfova et al. (2006)

4.4.3 Probiotic Side of Probiotic–Host Interactions

A growing body of literature suggests that there is a distinct difference between in vitro and in vivo gene expression profiles of probiotics. Apparently, the genes induced during colonization are dedicated for survival, adaptation, and growth of probiotics in the gut ecosystem. It is remarkable that many of these genes have also been found to be up-regulated in pathogenic organisms, revealing the existence of a common survival mechanism for probiotics and pathogens. Based on in vivo expression technology (IVET), it has been shown that three specific genes – xylose isomerase (*xylA*), peptide methionine sulfoxide reductase (*msrB*), a conserved hypothetical gene – were highly expressed in reconstituted *Lactobacillus*-free (RLF) mice inoculated with *L. reuteri* 100-23 (Walter et al. 2003). Whereas xylose isomerase is important for degradation of xylose for energy generation, peptide methionine sulfoxide reductase protects *L. reuteri* against oxidative damage. Using resolvase-based IVET, Bron et al. (2004) identified 72 genes in *L. plantarum* WCFS1 genome that were induced during colonization in the mouse gut. These genes play a role in sugar transport and utilization, acquisition and biosynthesis of nonsugar compounds, stress, and host interaction. With a microarray, Denou et al. (2007) analyzed the gene expression profile of *L. johnsonii* NCC533 in the gut segments of C3H/HeJ mice. The total number of genes highly expressed in the stomach, cecum, jejunum, and colon were 786, 391, 296, and 26, respectively. It is clearly evident from this study that probiotic genome expression depends on the local gut factors. Unfortunately, microarray expression data are lacking for probiotics of veterinary importance at this time. Future studies on this line undoubtedly would expand our knowledge on microbial gut colonization.

4.5 Effects of Probiotics on the Gut Microbiota

In addition to the host cells, probiotics interact with the bacteria present in the host gut. On one hand, probiotics may contribute to the growth of certain bacteria by supplying readily usable metabolites through the breakdown of complex nutrients. On the other hand, they may inhibit the growth of others, particularly pathogenic bacteria, by competing for nutrients and adhesion sites, production of acids and antimicrobial substances, and stimulation of intestinal immune response. Traditionally, the changes in the composition of the gut microbiota have been studied by culture techniques using selective agar media. Studies in animals concerning this aspect are limited, however. The results of some experimental animals studies involving the supplementation of probiotics are briefly presented here. Administration of a probiotic combination composed of *L. reuteri*, *Enterococcus faecium*, *B. animalis*, *Pediococcus acidilactici*, and *L. salivarius* has been shown to significantly increase *Bifidobacterium* spp., *Lactobacillus* spp., and Gram-positive cocci counts in 1-day-old chickens (Mountzouris et al. 2007). In contrast, a significant reduction

in the total concentrations of gut bacteria belonging to *Enterococcus* spp. and *E. faecalis* has been reported in 14-day-old piglets supplemented with *E. faecium* NCIMB10415 (Vahjen et al. 2007). Similarly, administration of *L. murinus* DPC6003 has been shown to reduce significantly the number of fecal Enterobacteriaceae in pigs (Gardiner et al. 2004). Probiotics can also be applied to reduce colonization of pathogenic bacteria and decrease the severity of diarrhea in animals. Indeed, Casey et al. (2007) observed a significant reduction in the fecal *Salmonella enterica* serovar typhimurium PT12 count at day 15 in pigs treated with a five-strain probiotic mixture composed of *L. murinus* DPC6002 and DPC6003, *L .pentosus* DPC6004, *L. salivaris* DPC6005, and *P. pentosaceus*.

It is well established that feedlot cattle are most important reservoirs of Shiga toxin-producing *Escherichia coli* including O157:H7 strain (Dowd 2007; Dowd et al. 2010; Dowd and Ishizaki 2006), the causative agent of hemorrhagic colitis and hemolytic-uremic syndrome in humans. About 30% of cattle are asymptomatic carriers and shed the bacterium in their feces, which serve as a source of carcass contamination during slaughter. With the aim of reducing the fecal shedding, several probiotic-based preharvest interventions have been tested with varying success. A direct-fed microbial (DFM) composed of two probiotic strains (i.e., *Streptococcus bovis* LCB6 and *Lactobacillus gallinarum* LCB 12) has been reported to inhibit completely the fecal shedding of *E. coli* O157 by increasing the production of volatile fatty acids, particularly acetic acid, in Holstein calves (Ohya et al. 2000). Recently, Stephens et al. (2007) used different *L. acidophilus* strains to examine their ability to reduce *E. coli* O157 in feces. In this study, 26.3% of control yearling steers shed *E. coli* O157 in feces, whereas the incidences in steers fed with *L. acidophilus* strains NP51, NP28, and NP-51-NP35 were estimated to be 13%, 11%, and 11%, respectively. Similarly, the feces from the controls had the highest concentration of *E. coli* O157 (3.2 log MPN/g) compared to the cattle supplemented with *L. acidophilus* strains NP51, NP28, and NP-51-NP35 (0.9, 1.1, and 1.7 log MPN/g of feces, respectively). It has been suggested by Peterson et al. (2007) that cattle supplemented with a daily dose of 10^9 CFU of *L. acidophilus* strains NP51 shed 35% less *E. coli* O157 than the controls over a 2-year period (Peterson et al. 2007). In contrast, a number of studies have shown that several other probiotic strains have no positive effects on fecal shedding of *E. coli* O157.

Unfortunately, more than 80% of gut bacteria cannot be cultured under current laboratory conditions, limiting assessment of the effects of probiotics on the gut microbiota. This drawback, however, has been overcome today to a large extent by employing molecular techniques. Examples of these techniques include 16S ribosomal RNA analysis, DGGE, RFLF, T-RFLP, and FISH. Using FISH, Gerard et al. (2008) studied the impact of *Lactobacillus* sp. no. 1-2673 on the *Atopobium* group, *Bacteroides-Prevotella* group, *Bifidobacterium* group, *Clostridium coccoides* group, *Faecalibacterium prusnitzii* group, Enterobacteria, and *Lactobacillus-Enterococcus* group in 4- and 19-day-old chickens. In this study, the probiotic did not change the composition of the cecal microbiota. On the other hand, the authors observed a highly diverse *Lactobacillus* group based on temporal temperature gradient gel electrophoresis only in 19-day-old chickens following

consumption of *Lactobacillus* sp. no. 1-2673. Similarly, using DGGE and T-RFLP, Fuentes reported a significant increase in the *Lactobacillus* spp. diversity in mice gut and feces administered *L. casei* and *L. plantarum* (Fuentes et al. 2008). However, there were no remarkable changes in the bacterial community in feces. Recently, Su et al. (2008) employed 16S rRNA-based PCR/DGGE and real-time PCR to investigate changes in the gut bacterial diversity of pigs following *L. sorbius* strain S1 supplementation. A specific band related to *Clostridium disporicum* and *Streptoccus suis* was found in the DGGE profiles of the control and treatment groups, respectively. However, the composition of the overall microbial community remained unchanged in both groups.

4.6   Effects of Probiotics in Bovines

Several probiotic strains have been shown to augment feed efficiency, body weight gain, and milk production and to decrease disease incidence and fecal shedding of *E.coli* in cattle. Table 4.4 summarizes some of the effects of probiotics on cattle performance. Often, the outcome of these studies is highly variable as several factors (such as age, gut microbiota, feed, strain of probiotic, dose and type of formulations) can modify the effects of probiotics. Based on previous studies in feedlot cattle, it has been suggested that probiotics increase daily gain and feed efficiency by 2.5–5.0% and 2.0%, respectively; and the average carcass weight gain is 6–7 kg (Krehbiel et al. 2003). Several researchers have demonstrated that the incidence of diarrhea can be decreased in calves by feeding them probiotics. For example, giving Holstein-Friesian calves a calf-specific multistrain probiotic (CSPB) containing six *Lactobacillus* strains markedly reduced the incidence (50%) and duration (58%) of diarrhea (Timmerman et al. 2005). Probiotics can also be employed to improve milk production in cows. In a recent experiment by Stein et al. (2006), Holstein cows fed *Propionibacterium* strain P169 at a dose of 6×10^{10} (low dose) or 6×10^{11} (high dose) cfu/cow had significantly higher milk production (7.1–8.5% FCM) than controls (4.0% FCM) during the 25-week study. However, the authors did not observe a significant difference between the high-dose P169 and low-dose P169 cows.

4.7   Effects of Probiotics in Swine

Environmental, psychological, and nutritional factors contribute to weaning-associated stress in piglets. Stress-induced changes include villous atrophy of small intestine and a decreased gut immune response, significantly increasing the susceptibility to infection caused by enteric pathogens. Several investigators have reported the beneficial effects of probiotics on recovery from postweaning stress. For example, administration of *Lactobacillus plantarum* Lq80 alone or with *Megasphaera elsdenii* iNP-001 increased the villous heights of the small intestine in piglets (Yoshida

Table 4.4 Positive effects of probiotics in cattle

Probiotic(s)	Dose	Type of animal	Reported probiotic effects	Reference
L. acidophilus (LA 51) P. freudenreichii (PF 24)	1×10^9 cfu/g feed (each strain)	Beef steers	Significantly decreased the fecal shedding of E. coli O157:H7	Tabe et al. (2008)
P. freudenreichii (NP 24) L. acidophilus (NP 51)	1×10^9 cfu/steer/day (NP24) 1×10^7 or 1×10^8 or 1×10^9 cfu/steer/day (NP 51)	Beef steers	Significantly increased the gain efficiency	Vasconcelos et al. (2008)
L. acidophilus W55, L. salivaris W57, L. paracasei spp. Paracasei W56, L. plantarum W59, L. lactis W58, and E. faecium W54	1×10^9 cfu/kg of BW	Holestein-Friesian calves	Significantly increased the daily gain, feed efficiency, and body weight; significantly decreased the incidence of diarrhea	Timmerman et al. (2005)
L. acidophilus NP 28 or NP51 or NP51-NP35	1×10^9 cfu/day (NP28 or NP51) 1×10^8 cfu/day (NP28 or NP51)	Beef steers	Significantly decreased the prevalence of E. coli O157	Stephens et al. (2007)
E. coli (strains 271, 786, and 797)	1×10^{10} cfu/calf	Holstein calves	Significantly decreased the fecal shedding of E. coli O26:H11 or O111:NM	Zhao et al. (2003)
Prevotella bryantii 25A	2×10^{11} cells/dose	Dairy cows	Significantly increased the total VFA concentration and milk fat percentage	Chiquette et al. (2008)
Dietzia ssp. C79793-74	2×10^{11} cfu; 3×10^{11} cfu; 4×10^{11} cfu	Dairy cows	Significantly prolonged the survivability; 37.5% of the animals are cured	Click and Van Kampen (2009)
Propionibacteria freudenreichii P169	6×10^{10} cfu/day	Dairy cows	Significantly increased the milk production, milk true protein content, and the pregnancy rate	de Ondarza and Seymour (2008)

et al. 2009). Similarly, villous height has been reported to be higher in piglets given supplements of *Pediococcus acidilactici* than in those of the control group (Di Giancamillo et al. 2008). Feeding *Bacillus cereus* var. toyoi to weaned piglets increased IgA levels in feces (Scharek et al. 2007) and reduced the incidence of diarrhea by 59% (Taras et al. 2005). Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause of devastating diarrhea in young pigs, leading to severe economic loss. A number of studies evaluated the effect of probiotics on lowering the incidence of *E. coli*-induced diarrhea. Indeed, the oral administration of *Bacillus subtilis* significantly decreased fecal scouring and mortality compared with that in the controls (Bhandari et al. 2008). Similar results were obtained in piglets fed *L. sobrius* (Konstantinov et al. 2008), *Enterococcus faecium* EK13 (Strompfova et al. 2006), *Enterococcus faecium* NCIMB 10415 (Taras et al. 2006), or *B. lactis* HN019 (Shu et al. 2001). Furthermore, growth performance can be improved by including *Lactobacillus sorbrius* (Konstantinov et al. 2008) or a probiotic containing *Bacillus lichniformis* and *Bacillus subtilis* spores in pig feed (Alexopoulos et al. 2004). Recent studies on probiotic effects in pigs are summarized in Table 4.5.

4.8 Effects of Probiotics in Poultry

Zhou et al. (2010) found a significantly higher final weight and daily weight gain in chickens fed *Bacillus coagulans* ZJU0616. The authors noted that the dosage of probiotic did not alter the outcome. In contrast, Jung et al. (2008) studied the effect of *Bifidobacterium lactis* D 300 on the performance of broiler chickens and reported that it did not improve body weight, feed intake, or the feed conversion ratio. These contrasting outcomes are not surprising as one of the determining factors for the performance is the strain of probiotics. Coccidiosis caused by *Eimeria* species is an economically important disease of poultry. To control the disease in chickens, various probiotics have been tested. Lee et al. (2007) reported that chickens treated with *Pediococcus acidilactici* had significantly reduced *E. acervulina* oocysts but not *E. tenella* oocysts in feces. The mechanism of a probiotic defense against coccidiosis remains to be explored. It has been shown that broiler chickens fed a probiotic mix composed of *Bacillus licheniformis* and *B. subtilis* had a significantly higher medial and lateral wall thickness of the tibiotarsi, percentage ash, and phosphorus content (Mutus et al. 2006); thus, probiotics may affect the development of bone. The effect of a probiotic product composed of *L. acidophilus*, *L. casei*, *B. bifidium*, and *Enterococccus faecium* on mucus dynamics in chickens was investigated by Smirnov et al. (2005), and the results revealed that there was a significant increase in mucin expression at both the RNA and protein level in the jejunum of the probiotic-supplemented group. It is clearly evident from the study by Haghighi et al. (2006) that a probiotic mix containing *L. acidophilus*, *B. bifidium*, and *Streptococcus faecalis* could significantly boost the level of serum and intestinal antibodies against tetanus toxoid (TT) and *Clostridium perfringens* α-toxin, intestinal IgA against BSA, and intestinal IgG antibodies against TT in chickens. Table 4.6 summarizes the latest findings of the effects of probiotics on poultry growth, performance, and health.

Table 4.6 Positive effects of probiotics in poultry

Probiotic(s)	Dose	Type of birds	Reported probiotic effects	Reference
Bacillus subtilis	50 mg/kg feed	Ross chicks	Significantly increased the body weight gain, feed conversion, and the level of antibodies against sheep red blood cell (SRBC) and Newcastle disease virus	Khaksefidi and Ghoorchi (2006)
L. reuteri DSM 16350, *E. facium* DSM 16211, *B. animalis* DSM 16284, *Pediococcus acidilactici* DSM 16210, *L. salivarius* DSM 16351	1×10^8 cfu/kg of feed	Cobb broilers	Significantly increased the body weight gain and feed conversion	Mountzouris et al. (2010)
L. plantarum (SRP, YJG), *L.salivaris* (MA), *L.acidophilus* (LA, GA, SJ)	1×10^9 cfu/ml; 5 ml/kg	Arbor Acres broilers	YJG significantly increased the average daily gain and feed intake; MA, LA, and SRP significantly increased Roche color fan scores of the shank skin	Zhu et al. (2009)
L. casei, L.acidophilus, B.thermophilum, E.faecium	1×10^8 cfu/g	Broiler chickens	Significantly increased the intestinal muscle thickness, villous height, and perimeter; significantly decreased bacterial colonization	Chichlowski et al. (2007)
L. reuteri, E. faecium, B. animalis, P. acidilactici and *L. salivarius*	2×10^9 cfu/kg diet	Cobb broilers	Significantly reduced the incidence of *Salmonella enteritidis* infection	Mountzouris et al. (2009)
E.faecium, P. acidilactici, B. animalis, and *L. reuteri*	1.3×10^{11} cfu or 1.4×10^{12} cfu	Ross × Ross chicks	Significantly reduced the necrotic enteritis lesions, mortality, and the concentration of *C. perfringens*	McReynolds et al. (2009)
L. reuteri, E. faecium, B. animalis, P. acidilactici	1.7×10^8 cfu/kg diet	Ross × Ross chicks	Reduced the extend and duration of LPS-induced anorexic effects	Jiang et al. (2010)
B. longum PCB 133	1×10^8 cfu/day	Broiler chickens	Significantly decreased fecal shedding of *C. jejuni*	Santini et al. (2010)

4.9   Fecal Bacteriotherapy and Fecal Transplants

Taking a single bacterium or a group of bacteria isolated and propagated under laboratory conditions represents a logical and controlled commercial model. It is well known that after propagation in the laboratory many types of bacteria lose inherent capabilities and even entire genes that might enhance their ability to be optimized as a therapeutic agent. The notion of bacteriotherapy and fecal transplantation is not a novel concept and has been practiced in various forms for centuries. Taking feces from healthy animals and feeding it to sick animals may seem outrageous to some, but it is a poorly publicized practice in many parts of the world. There is a resurgence of bacteriotherapy in modern medicine (Floch 2010; Khoruts et al. 2010; Marteau et al. 2009; Russell et al. 2010), and historical accounts that go back beyond 1885 (Journal 1885) set the stage for what may be the future of probiotics. The consideration that we can take entire gut populations with their existing community interactions and stabilities from a healthy high-production animal and transfer such a benefit to a sick or low-efficiency animal and effectively monitor this at the microbiome (such as with the bTEFAP method) and at the physiological level is an attractive option for future consideration. Replacing a dysfunctional microbiome with a complete and functional microbiome inherently and ecologically is more logical than taking a laboratory strain of bacteria and hoping it can survive and thrive in the extreme and competitive environment of the gut. Philosophy and postulation aside, the future of probiotics will develop into a more ecologically sound approach and foundation driven by modern science.

4.10   Conclusion and Future Directions

Probiotics and their effects on gut health and the existing microbiome have entered the age of modern genomics. Future work in sequencing genomes of notable animal-related probiotic strains, metagenomic studies to evaluate the effects of probiotic therapies, and microbiome efforts will continue to shed light on how we can best identify and optimize probiotic therapies and isolates. Further efforts on isolating new types of bacteria should be considered. As we have noted, only a small percentage of bacteria can be propagated in the laboratory. Many important probiotic microbes may still be undiscovered and their potential untapped because we do not have sufficient technologies to enable their propagation. Future controlled efforts in whole microbe community (microbiome) transplantation (taking the microbiome intact from highly efficient, healthy animals and transplanting it into sick or low-efficiency animals) may represent a coming evolution (or resurgence) in the field of probiotics, especially as our ability to evaluate entire metagenomes (the cumulative genome of an entire population) is improving. Host genomic interactions with the microbiome is only now beginning to be elucidated; and, again, as modern scientific methods become more cost-effective and powerful we will reveal the hidden secrets that continue to enhance our ability to cooperate with our microbial partners.

References

Alexopoulos, C., I.E. Georgoulakis, A. Tzivara, S.K. Kritas, A. Siochu, and S.C. Kyriakis. 2004. Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores, on the health status and performance of sows and their litters. *Journal of Animal Physiology and Animal Nutrition* 88: 381–392.

Altermann, E., W.M. Russell, M.A. Azcarate-Peril, R. Barrangou, B.L. Buck, O. McAuliffe, N. Souther, A. Dobson, T. Duong, M. Callanan, S. Lick, A. Hamrick, R. Cano, and T.R. Klaenhammer. 2005. Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proceedings of the National Academy of Sciences of the United States of America* 102: 3906–3912.

Azcarate-Peril, M.A., E. Altermann, Y.J. Goh, R. Tallon, R.B. Sanozky-Dawes, E.A. Pfeiler, S. O'Flaherty, B.L. Buck, A. Dobson, T. Duong, M.J. Miller, R. Barrangou, and T.R. Klaenhammer. 2008. Analysis of the genome sequence of *Lactobacillus gasseri* ATCC 33323 reveals the molecular basis of an autochthonous intestinal organism. *Applied and Environmental Microbiology* 74: 4610–4625.

Bailey, M.T., S.E. Dowd, N.M. Parry, J.D. Galley, D.B. Schauer, and M. Lyte. 2010. Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by *Citrobacter rodentium*. *Infection and Immunity* 78: 1509–1519.

Barrangou, R., E.P. Briczinski, L.L. Traeger, J.R. Loquasto, M. Richards, P. Horvath, A.C. Coute-Monvoisin, G. Leyer, S. Rendulic, J.L. Steele, J.R. Broadbent, T. Oberg, E.G. Dudley, S. Schuster, D.A. Romero, and R.F. Roberts. 2009. Comparison of the complete genome sequences of *Bifidobacterium animalis* subsp. lactis DSM 10140 and Bl-04. *Journal of Bacteriology* 191: 4144–4151.

Bhandari, S.K., B. Xu, C.M. Nyachoti, D.W. Giesting, and D.O. Krause. 2008. Evaluation of alternatives to antibiotics using an *Escherichia coli* K88+ model of piglet diarrhea: Effects on gut microbial ecology. *Journal of Animal Science* 86: 836–847.

Brisbin, J.T., H. Zhou, J. Gong, P. Sabour, M.R. Akbari, H.R. Haghighi, H. Yu, A. Clarke, A.J. Sarson, and S. Sharif. 2008. Gene expression profiling of chicken lymphoid cells after treatment with *Lactobacillus acidophilus* cellular components. *Developmental and Comparative Immunology* 32: 563–574.

Bron, P.A., C. Grangette, A. Mercenier, W.M. de Vos, and M. Kleerebezem. 2004. Identification of *Lactobacillus plantarum* genes that are induced in the gastrointestinal tract of mice. *Journal of Bacteriology* 186: 5721–5729.

Callaway, T.R., S.E. Dowd, R.D. Wolcott, Y. Sun, J.L. McReynolds, T.S. Edrington, J.A. Byrd, R.C. Anderson, N. Krueger, and D.J. Nisbet. 2009. Evaluation of the bacterial diversity in cecal contents of laying hens fed various molting diets by using bacterial tag-encoded FLX amplicon pyrosequencing. *Poultry Science* 88: 298–302.

Callaway, T.R., S.E. Dowd, T.S. Edrington, R.C. Anderson, N. Krueger, N. Bauer, P.J. Kononoff, and D.J. Nisbet. 2010. Evaluation of bacterial diversity in the rumen and feces of cattle fed different levels of dried distillers grains plus solubles using bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *Journal of Animal Science* 88(12): 3977–3983.

Casey, P.G., G.E. Gardiner, G. Casey, B. Bradshaw, P.G. Lawlor, P.B. Lynch, F.C. Leonard, C. Stanton, R.P. Ross, G.F. Fitzgerald, and C. Hill. 2007. A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with *Salmonella enterica* Serovar Typhimurium. *Applied and Environmental Microbiology* 73: 1858–1863.

Chichlowski, M., W.J. Croom, F.W. Edens, B.W. McBride, R. Qiu, C.C. Chiang, L.R. Daniel, G.B. Havenstein, and M.D. Koci. 2007. Microarchitecture and spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac, and salinomycin. *Poultry Science* 86: 1121–1132.

Chiquette, J., M.J. Allison, and M.A. Rasmussen. 2008. Prevotella bryantii 25A used as a probiotic in early-lactation dairy cows: Effect on ruminal fermentation characteristics, milk production, and milk composition. *Journal of Dairy Science* 91: 3536–3543.

Claesson, M.J., Y. Li, S. Leahy, C. Canchaya, J.P. van Pijkeren, A.M. Cerdeno-Tarraga, J. Parkhill, S. Flynn, G.C. O'Sullivan, J.K. Collins, D. Higgins, F. Shanahan, G.F. Fitzgerald, D. van Sinderen, and P.W. O'Toole. 2006. Multireplicon genome architecture of *Lactobacillus salivarius*. *Proceedings of the National Academy of Sciences of the United States of America* 103: 6718–6723.

Click, R.E., and C.L. Van Kampen. 2009. Short communication: Progression of Johne's disease curtailed by a probiotic. *Journal of Dairy Science* 92: 4846–4851.

Davis, M.E., T. Parrott, D.C. Brown, B.Z. de Rodas, Z.B. Johnson, C.V. Maxwell, and T. Rehberger. 2008. Effect of a Bacillus-based direct-fed microbial feed supplement on growth performance and pen cleaning characteristics of growing-finishing pigs. *Journal of Animal Science* 86: 1459–1467.

de Ondarza, M.B., and W.M. Seymour. 2008. Effect of Propionibacteria supplementation on yield of milk and milk components of dairy cows. *The Professional Animal Scientist* 24: 254–259.

Denou, E., B. Berger, C. Barretto, J.M. Panoff, F. Arigoni, and H. Brussow. 2007. Gene expression of commensal *Lactobacillus johnsonii* strain NCC533 during in vitro growth and in the murine gut. *Journal of Bacteriology* 189: 8109–8119.

Desai, A.R., K.M. Musil, A.P. Carr, and J.E. Hill. 2009. Characterization and quantification of feline fecal microbiota using cpn60 sequence-based methods and investigation of animal-to-animal variation in microbial population structure. *Veterinary Microbiology* 137: 120–128.

Dethlefsen, L., P.B. Eckburg, E.M. Bik, and D.A. Relman. 2006. Assembly of the human intestinal microbiota. *Trends in Ecology and Evolution* 21: 517–523.

Dethlefsen, L., S. Huse, M.L. Sogin, and D.A. Relman. 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biology* 6: e280.

Di Giancamillo, A., F. Vitari, G. Savoini, V. Bontempo, C. Bersani, V. Dell'Orto, and C. Domeneghini. 2008. Effects of orally administered probiotic *Pediococcus acidilactici* on the small and large intestine of weaning piglets. A qualitative and quantitative micro-anatomical study. *Histology and Histopathology* 23: 651–664.

Dowd, S.E. 2007. *Escherichia coli* O157:H7 gene expression in the presence of catecholamine norepinephrine. *FEMS Microbiology Letters* 273: 214–223.

Dowd, S.E., and H. Ishizaki. 2006. Microarray based comparison of two *Escherichia coli* O157:H7 lineages. *BMC Microbiology* 6: 30.

Dowd, S.E., T.R. Callaway, R.D. Wolcott, Y. Sun, T. McKeehan, R.G. Hagevoort, and T.S. Edrington. 2008a. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiology* 8: 125.

Dowd, S.E., Y. Sun, P.R. Secor, D.D. Rhoads, B.M. Wolcott, G.A. James, and R.D. Wolcott. 2008b. Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. *BMC Microbiology* 8: 43.

Dowd, S.E., Y. Sun, R.D. Wolcott, A. Domingo, and J.A. Carroll. 2008c. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: Bacterial diversity in the ileum of newly weaned Salmonella-infected pigs. *Foodborne Pathogens and Disease* 5: 459–472.

Dowd, S.E., T.L. Crippen, Y. Sun, V. Gontcharova, E. Youn, A. Muthaiyan, R.D. Wolcott, T.R. Callaway, and S.C. Ricke. 2010. Microarray analysis and draft genomes of two *Escherichia coli* O157:H7 lineage II cattle isolates FRIK966 and FRIK2000 investigating lack of shiga toxin expression. *Foodborne Pathogens and Disease* 7: 763–773.

Floch, M.H. 2010. Fecal bacteriotherapy, fecal transplant, and the microbiome. *Journal of Clinical Gastroenterology* 44: 529–530.

Forney, L.J., X. Zhou, and C.J. Brown. 2004. Molecular microbial ecology: Land of the one-eyed king. *Current Opinion in Microbiology* 7: 210–220.

Fuentes, S., M. Egert, M. Jimenez-Valera, A. Ramos-Cormenzana, A. Ruiz-Bravo, H. Smidt, and M. Monteoliva-Sanchez. 2008. Administration of *Lactobacillus casei* and *Lactobacillus plantarum* affects the diversity of murine intestinal lactobacilli, but not the overall bacterial community structure. *Research in Microbiology* 159: 237–243.

Gardiner, G.E., P.G. Casey, G. Casey, P.B. Lynch, P.G. Lawlor, C. Hill, G.F. Fitzgerald, C. Stanton, and R.P. Ross. 2004. Relative ability of orally administered *Lactobacillus murinus* to predominate and persist in the porcine gastrointestinal tract. *Applied and Environmental Microbiology* 70: 1895–1906.

Gerard, P., C. Brezillon, F. Quere, A. Salmon, and S. Rabot. 2008. Characterization of cecal microbiota and response to an orally administered lactobacillus probiotic strain in the broiler chicken. *Journal of Molecular Microbiology and Biotechnology* 14: 115–122.

Ghorbani, G.R., D.P. Morgavi, K.A. Beauchemin, and J.A. Leedle. 2002. Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. *Journal of Animal Science* 80: 1977–1985.

Granato, D., G.E. Bergonzelli, R.D. Pridmore, L. Marvin, M. Rouvet, and I.E. Corthesy-Theulaz. 2004. Cell surface-associated elongation factor Tu mediates the attachment of *Lactobacillus johnsonii* NCC533 (La1) to human intestinal cells and mucins. *Infection and Immunity* 72: 2160–2169.

Haghighi, H.R., J. Gong, C.L. Gyles, M.A. Hayes, H. Zhou, B. Sanei, J.R. Chambers, and S. Sharif. 2006. Probiotics stimulate production of natural antibodies in chickens. *Clinical and Vaccine Immunology* 13: 975–980.

Janeczko, S., D. Atwater, E. Bogel, A. Greiter-Wilke, A. Gerold, M. Baumgart, H. Bender, P.L. McDonough, S.P. McDonough, R.E. Goldstein, and K.W. Simpson. 2008. The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. *Veterinary Microbiology* 128: 178–193.

Jiang, Z., G. Schatzmayr, M. Mohnl, and T.J. Applegate. 2010. Net effect of an acute phase response – Partial alleviation with probiotic supplementation. *Poultry Science* 89: 28–33.

Journal, T.B.M. 1885. Bacteriotherapy. *British Medical Journal* 28: 1.

Jung, S.J., R. Houde, B. Baurhoo, X. Zhao, and B.H. Lee. 2008. Effects of galacto-oligosaccharides and a Bifidobacteria lactis-based probiotic strain on the growth performance and fecal microflora of broiler chickens. *Poultry Science* 87: 1694–1699.

Kankainen, M., L. Paulin, S. Tynkkynen, I. von Ossowski, J. Reunanen, P. Partanen, R. Satokari, S. Vesterlund, A.P. Hendrickx, S. Lebeer, S.C. De Keersmaecker, J. Vanderleyden, T. Hamalainen, S. Laukkanen, N. Salovuori, J. Ritari, E. Alatalo, R. Korpela, T. Mattila-Sandholm, A. Lassig, K. Hatakka, K.T. Kinnunen, H. Karjalainen, M. Saxelin, K. Laakso, A. Surakka, A. Palva, T. Salusjarvi, P. Auvinen, and W.M. de Vos. 2009. Comparative genomic analysis of Lactobacillus rhamnosus GG reveals pili containing a human-mucus binding protein. *Proceedings of the National Academy of Sciences of the United States of America* 106: 17193–17198.

Khaksefidi, A., and T. Ghoorchi. 2006. Effect of probiotic on performance and immunocompetence in broiler chicks. *The Journal of Poultry Science* 43: 296–300.

Khoruts, A., J. Dicksved, J.K. Jansson, and M.J. Sadowsky. 2010. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea. *Journal of Clinical Gastroenterology* 44: 354–360.

Kim, J.F., H. Jeong, D.S. Yu, S.H. Choi, C.G. Hur, M.S. Park, S.H. Yoon, D.W. Kim, G.E. Ji, H.S. Park, and T.K. Oh. 2009. Genome sequence of the probiotic bacterium Bifidobacterium animalis subsp. lactis AD011. *Journal of Bacteriology* 191: 678–679.

Kleerebezem, M., J. Boekhorst, R. van Kranenburg, D. Molenaar, O.P. Kuipers, R. Leer, R. Tarchini, S.A. Peters, H.M. Sandbrink, M.W. Fiers, W. Stiekema, R.M. Lankhorst, P.A. Bron, S.M. Hoffer, M.N. Groot, R. Kerkhoven, M. de Vries, B. Ursing, W.M. de Vos, and R.J. Siezen. 2003. Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proceedings of the National Academy of Sciences of the United States of America* 100: 1990–1995.

Konstantinov, S.R., H. Smidt, A.D. Akkermans, L. Casini, P. Trevisi, M. Mazzoni, S. De Filippi, P. Bosi, and W.M. de Vos. 2008. Feeding of *Lactobacillus sobrius* reduces *Escherichia coli* F4 levels in the gut and promotes growth of infected piglets. *FEMS Microbiology Ecology* 66: 599–607.

Krehbiel, C.R., S.R. Rust, G. Zhang, and S.E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *Journal of Animal Science* 81: E120–E132.

Lee, S., H.S. Lillehoj, D.W. Park, Y.H. Hong, and J.J. Lin. 2007. Effects of Pediococcus- and Saccharomyces-based probiotic (MitoMax) on coccidiosis in broiler chickens. *Comparative Immunology, Microbiology and Infectious Diseases* 30: 261–268.

Lee, J.H., V.N. Karamychev, S.A. Kozyavkin, D. Mills, A.R. Pavlov, N.V. Pavlova, N.N. Polouchine, P.M. Richardson, V.V. Shakhova, A.I. Slesarev, B. Weimer, and D.J. O'Sullivan. 2008. Comparative genomic analysis of the gut bacterium Bifidobacterium longum reveals loci susceptible to deletion during pure culture growth. *BMC Genomics* 9: 247.

Lin, P.W., T.R. Nasr, A.J. Berardinelli, A. Kumar, and A.S. Neish. 2008. The probiotic Lactobacillus GG may augment intestinal host defense by regulating apoptosis and promoting cytoprotective responses in the developing murine gut. *Pediatric Research* 64: 511–516.

Lipski, A., U. Friedrich, and K. Altendorf. 2001. Application of rRNA-targeted oligonucleotide probes in biotechnology. *Applied Microbiology and Biotechnology* 56: 40–57.

Lubbs, D.C., B.M. Vester, N.D. Fastinger, and K.S. Swanson. 2009. Dietary protein concentration affects intestinal microbiota of adult cats: A study using DGGE and qPCR to evaluate differences in microbial populations in the feline gastrointestinal tract. *Journal of Animal Physiology and Animal Nutrition* 93: 113–121.

Mack, D.R., S. Ahrne, L. Hyde, S. Wei, and M.A. Hollingsworth. 2003. Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro. *Gut* 52: 827–833.

Marteau, P., H. Sokol, X. Dray, and P. Seksik. 2009. Bacteriotherapy for inflammatory bowel disease: Therapeutic tool and/or pharmacological vectors? *Gastroenterologie Clinique et Biologique* 33(Suppl 3): S228–S234.

Matsuguchi, T., A. Takagi, T. Matsuzaki, M. Nagaoka, K. Ishikawa, T. Yokokura, and Y. Yoshikai. 2003. Lipoteichoic acids from Lactobacillus strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2. *Clinical and Diagnostic Laboratory Immunology* 10: 259–266.

McReynolds, J., C. Waneck, J. Byrd, K. Genovese, S. Duke, and D. Nisbet. 2009. Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. *Poultry Science* 88: 2075–2080.

Morita, H., H. Toh, S. Fukuda, H. Horikawa, K. Oshima, T. Suzuki, M. Murakami, S. Hisamatsu, Y. Kato, T. Takizawa, H. Fukuoka, T. Yoshimura, K. Itoh, D.J. O'Sullivan, L.L. McKay, H. Ohno, J. Kikuchi, T. Masaoka, and M. Hattori. 2008. Comparative genome analysis of *Lactobacillus reuteri* and *Lactobacillus fermentum* reveal a genomic island for reuterin and cobalamin production. *DNA Research* 15: 151–161.

Morita, H., H. Toh, K. Oshima, M. Murakami, T.D. Taylor, S. Igimi, and M. Hattori. 2009. Complete genome sequence of the probiotic *Lactobacillus rhamnosus* ATCC 53103. *Journal of Bacteriology* 191: 7630–7631.

Mountzouris, K.C., P. Tsirtsikos, E. Kalamara, S. Nitsch, G. Schatzmayr, and K. Fegeros. 2007. Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science* 86: 309–317.

Mountzouris, K.C., C. Balaskas, I. Xanthakos, A. Tzivinikou, and K. Fegeros. 2009. Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with Salmonella enteritidis. *British Poultry Science* 50: 467–478.

Mountzouris, K.C., P. Tsitrsikos, I. Palamidi, A. Arvaniti, M. Mohnl, G. Schatzmayr, and K. Fegeros. 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science* 89: 58–67.

Mutus, R., N. Kocabagli, M. Alp, N. Acar, M. Eren, and S.S. Gezen. 2006. The effect of dietary probiotic supplementation on tibial bone characteristics and strength in broilers. *Poultry Science* 85: 1621–1625.

Netherwood, T., H.J. Gilbert, D.S. Parker, and A.G. O'Donnell. 1999. Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. *Applied and Environmental Microbiology* 65: 5134–5138.

Ohya, T., T. Marubashi, and H. Ito. 2000. Significance of fecal volatile fatty acids in shedding of *Escherichia coli* O157 from calves: Experimental infection and preliminary use of a probiotic product. *The Journal of Veterinary Medical Science* 62: 1151–1155.

Ojala, T., V. Kuparinen, J.P. Koskinen, E. Alatalo, L. Holm, P. Auvinen, S. Edelman, B. Westerlund-Wikstrom, T.K. Korhonen, L. Paulin, and M. Kankainen. 2010. Genome sequence of Lactobacillus crispatus ST1. *Journal of Bacteriology* 192: 3547–3548.

Panigrahi, P., G.T. Braileanu, H. Chen, and O.C. Stine. 2007. Probiotic bacteria change *Escherichia coli*-induced gene expression in cultured colonocytes: Implications in intestinal pathophysiology. *World Journal of Gastroenterology* 13: 6370–6378.

Peterson, R.E., T.J. Klopfenstein, G.E. Erickson, J. Folmer, S. Hinkley, R.A. Moxley, and D.R. Smith. 2007. Effect of *Lactobacillus acidophilus* strain NP51 on *Escherichia coli* O157:H7 fecal shedding and finishing performance in beef feedlot cattle. *Journal of Food Protection* 70: 287–291.

Pollmann, M., M. Nordhoff, A. Pospischil, K. Tedin, and L.H. Wieler. 2005. Effects of a probiotic strain of *Enterococcus faecium* on the rate of natural chlamydia infection in swine. *Infection and Immunity* 73: 4346–4353.

Pridmore, R.D., B. Berger, F. Desiere, D. Vilanova, C. Barretto, A.C. Pittet, M.C. Zwahlen, M. Rouvet, E. Altermann, R. Barrangou, B. Mollet, A. Mercenier, T. Klaenhammer, F. Arigoni, and M.A. Schell. 2004. The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533. *Proceedings of the National Academy of Sciences of the United States of America* 101: 2512–2517.

Ritchie, L., J. Steiner, and J.S. Suchodolski. 2008. Assessment of microbial diversity along the feline intestinal tract using 16S rRNA gene analysis. *FEMS Microbiology Ecology* 66: 590–598.

Russell, G., J. Kaplan, M. Ferraro, and I.C. Michelow. 2010. Fecal bacteriotherapy for relapsing *Clostridium difficile* infection in a child: A proposed treatment protocol. *Pediatrics* 126: e239–e242.

Ryan, S.M., G.F. Fitzgerald, and D. van Sinderen. 2005. Transcriptional regulation and characterization of a novel beta-fructofuranosidase-encoding gene from *Bifidobacterium breve* UCC2003. *Applied and Environmental Microbiology* 71: 3475–3482.

Santini, C., L. Baffoni, F. Gaggia, M. Granata, R. Gasbarri, D. Di Gioia, and B. Biavati. 2010. Characterization of probiotic strains: An application as feed additives in poultry against *Campylobacter jejuni*. *International Journal of Food Microbiology* 141(Suppl. 1): S98–S108.

Scharek, L., J. Guth, M. Filter, and M.F. Schmidt. 2007. Impact of the probiotic bacteria *Enterococcus faecium* NCIMB 10415 (SF68) and *Bacillus cereus* var. toyoi NCIMB 40112 on the development of serum IgG and faecal IgA of sows and their piglets. *Archives of Animal Nutrition* 61: 223–234.

Schell, M.A., M. Karmirantzou, B. Snel, D. Vilanova, B. Berger, G. Pessi, M.C. Zwahlen, F. Desiere, P. Bork, M. Delley, R.D. Pridmore, and F. Arigoni. 2002. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proceedings of the National Academy of Sciences of the United States of America* 99: 14422–14427.

Schlee, M., J. Wehkamp, A. Altenhoefer, T.A. Oelschlaeger, E.F. Stange, and K. Fellermann. 2007. Induction of human beta-defensin 2 by the probiotic *Escherichia coli* Nissle 1917 is mediated through flagellin. *Infection and Immunity* 75: 2399–2407.

Sela, D.A., J. Chapman, A. Adeuya, J.H. Kim, F. Chen, T.R. Whitehead, A. Lapidus, D.S. Rokhsar, C.B. Lebrilla, J.B. German, N.P. Price, P.M. Richardson, and D.A. Mills. 2008. The genome sequence of *Bifidobacterium longum* subsp. infantis reveals adaptations for milk utilization within the infant microbiome. *Proceedings of the National Academy of Sciences of the United States of America* 105: 18964–18969.

Sghir, A., G. Gramet, A. Suau, V. Rochet, P. Pochart, and J. Dore. 2000. Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. *Applied and Environmental Microbiology* 66: 2263–2266.

Shima, T., K. Fukushima, H. Setoyama, A. Imaoka, S. Matsumoto, T. Hara, K. Suda, and Y. Umesaki. 2008. Differential effects of two probiotic strains with different bacteriological

properties on intestinal gene expression, with special reference to indigenous bacteria. *FEMS Immunology and Medical Microbiology* 52: 69–77.

Shu, Q., F. Qu, and H.S. Gill. 2001. Probiotic treatment using *Bifidobacterium lactis* HN019 reduces weanling diarrhea associated with rotavirus and *Escherichia coli* infection in a piglet model. *Journal of Pediatric Gastroenterology and Nutrition* 33: 171–177.

Siggers, R.H., J. Siggers, M. Boye, T. Thymann, L. Molbak, T. Leser, B.B. Jensen, and P.T. Sangild. 2008. Early administration of probiotics alters bacterial colonization and limits diet-induced gut dysfunction and severity of necrotizing enterocolitis in preterm pigs. *The Journal of Nutrition* 138: 1437–1444.

Silverman, G.A., P.I. Bird, R.W. Carrell, F.C. Church, P.B. Coughlin, P.G. Gettins, J.A. Irving, D.A. Lomas, C.J. Luke, R.W. Moyer, P.A. Pemberton, E. Remold-O'Donnell, G.S. Salvesen, J. Travis, and J.C. Whisstock. 2001. The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *The Journal of Biological Chemistry* 276: 33293–33296.

Smirnov, A., R. Perez, E. Amit-Romach, D. Sklan, and Z. Uni. 2005. Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. *The Journal of Nutrition* 135: 187–192.

Sonnenburg, J.L., C.T. Chen, and J.I. Gordon. 2006. Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. *PLoS Biology* 4: e413.

Stein, D.R., D.T. Allen, E.B. Perry, J.C. Bruner, K.W. Gates, T.G. Rehberger, K. Mertz, D. Jones, and L.J. Spicer. 2006. Effects of feeding propionibacteria to dairy cows on milk yield, milk components, and reproduction. *Journal of Dairy Science* 89: 111–125.

Stephens, T.P., G.H. Loneragan, L.M. Chichester, and M.M. Brashears. 2007. Prevalence and enumeration of *Escherichia coli* O157 in steers receiving various strains of Lactobacillus-based direct-fed microbials. *Journal of Food Protection* 70: 1252–1255.

Strompfova, V., M. Marcinakova, M. Simonova, S. Gancarcikova, Z. Jonecova, L. Scirankova, J. Koscova, V. Buleca, K. Cobanova, and A. Laukova. 2006. Enterococcus faecium EK13 – An enterocin a-producing strain with probiotic character and its effect in piglets. *Anaerobe* 12: 242–248.

Su, Y., W. Yao, O.N. Perez-Gutierrez, H. Smidt, and W.Y. Zhu. 2008. 16S ribosomal RNA-based methods to monitor changes in the hindgut bacterial community of piglets after oral administration of *Lactobacillus sobrius* S1. *Anaerobe* 14: 78–86.

Suchodolski, J.S., J. Camacho, and J.M. Steiner. 2008. Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. *FEMS Microbiology Ecology* 66: 567–578.

Suchodolski, J.S., S.E. Dowd, E. Westermarck, J.M. Steiner, R.D. Wolcott, T. Spillmann, and J.A. Harmoinen. 2009. The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rRNA gene sequencing. *BMC Microbiology* 9: 210.

Tabe, E.S., J. Oloya, D.K. Doetkott, M.L. Bauer, P.S. Gibbs, and M.L. Khaitsa. 2008. Comparative effect of direct-fed microbials on fecal shedding of Escherichia coli O157:H7 and Salmonella in naturally infected feedlot cattle. *Journal of Food Protection* 71: 539–544.

Takeda, K., T. Kaisho, and S. Akira. 2003. Toll-like receptors. *Annual Review of Immunology* 21: 335–376.

Tao, Y., K.A. Drabik, T.S. Waypa, M.W. Musch, J.C. Alverdy, O. Schneewind, E.B. Chang, and E.O. Petrof. 2006. Soluble factors from Lactobacillus GG activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells. *American Journal of Physiology Cell Physiology* 290: C1018–C1030.

Taras, D., W. Vahjen, M. Macha, and O. Simon. 2005. Response of performance characteristics and fecal consistency to long-lasting dietary supplementation with the probiotic strain *Bacillus cereus* var. toyoi to sows and piglets. *Archives of Animal Nutrition* 59: 405–417.

Taras, D., W. Vahjen, M. Macha, and O. Simon. 2006. Performance, diarrhea incidence, and occurrence of *Escherichia coli* virulence genes during long-term administration of a probiotic Enterococcus faecium strain to sows and piglets. *Journal of Animal Science* 84: 608–617.

Timmerman, H.M., L. Mulder, H. Everts, D.C. van Espen, E. van der Wal, G. Klaassen, S.M. Rouwers, R. Hartemink, F.M. Rombouts, and A.C. Beynen. 2005. Health and growth of veal calves fed milk replacers with or without probiotics. *Journal of Dairy Science* 88: 2154–2165.

Vahjen, W., D. Taras, and O. Simon. 2007. Effect of the probiotic *Enterococcus faecium* NCIMB10415 on cell numbers of total Enterococcus spp., *E. faecium* and *E. faecalis* in the intestine of piglets. *Current Issues in Intestinal Microbiology* 8: 1–7.

Vasconcelos, J.T., N.A. Elam, M.M. Brashears, and M.L. Galyean. 2008. Effects of increasing dose of live cultures of *Lactobacillus acidophilus* (Strain NP 51) combined with a single dose of *Propionibacterium freudenreichii* (Strain NP 24) on performance and carcass characteristics of finishing beef steers. *Journal of Animal Science* 86: 756–762.

Walter, J., N.C. Heng, W.P. Hammes, D.M. Loach, G.W. Tannock, and C. Hertel. 2003. Identification of *Lactobacillus reuteri* genes specifically induced in the mouse gastrointestinal tract. *Applied and Environmental Microbiology* 69: 2044–2051.

Wang, A., H. Yu, X. Gao, X. Li, and S. Qiao. 2009a. Influence of *Lactobacillus fermentum* I5007 on the intestinal and systemic immune responses of healthy and *E. coli* challenged piglets. *Antonie Van Leeuwenhoek* 96: 89–98.

Wang, M., G. Zhang, X. Han, L. Yao, Y. Zhou, and Y. Jiang. 2009b. Expression profile analysis of intestinal Caco-2 cells treated with *Lactobacillus acidophilus* NCFM. *Wei Sheng Wu Xue Bao* 49: 1247–1252.

Wegmann, U., K. Overweg, N. Horn, A. Goesmann, A. Narbad, M.J. Gasson, and C. Shearman. 2009. Complete genome sequence of *Lactobacillus johnsonii* FI9785, a competitive exclusion agent against pathogens in poultry. *Journal of Bacteriology* 191: 7142–7143.

Weiss, G., S. Rasmussen, L.H. Zeuthen, B.N. Nielsen, H. Jarmer, L. Jespersen, and H. Frokiaer. 2010. *Lactobacillus acidophilus* induces virus immune defence genes in murine dendritic cells by a Toll-like receptor-2-dependent mechanism. *Immunology* 131(2): 268–281.

Yan, F., H. Cao, T.L. Cover, R. Whitehead, M.K. Washington, and D.B. Polk. 2007. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* 132: 562–575.

Yoshida, Y., T. Tsukahara, and K. Ushida. 2009. Oral administration of *Lactobacillus plantarum* Lq80 and Megasphaera elsdenii iNP-001 induces efficient recovery from mucosal atrophy in the small and the large intestines of weaning piglets. *Animal Science Journal* 80: 709–715.

Zhao, T., S. Tkalcic, M.P. Doyle, B.G. Harmon, C.A. Brown, and P. Zhao. 2003. Pathogenicity of enterohemorrhagic *Escherichia coli* in neonatal calves and evaluation of fecal shedding by treatment with probiotic *Escherichia coli*. *Journal of Food Protection* 66: 924–930.

Zhou, X., Y. Wang, Q. Gu, and W. Li. 2010. Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science* 89: 588–593.

Zhu, N.H., R.J. Zhang, H. Wu, and B. Zhang. 2009. Effects of *Lactobacillus* cultures on growth performance, xanthophyll deposition, and color of the meat and skin of broilers. *Journal of Applied Poultry Science* 18: 570–578.

Zoetendal, E.G., B. Cheng, S. Koike, and R.I. Mackie. 2004. Molecular microbial ecology of the gastrointestinal tract: From phylogeny to function. *Current Issues in Intestinal Microbiology* 5: 31–47.

Zyrek, A.A., C. Cichon, S. Helms, C. Enders, U. Sonnenborn, and M.A. Schmidt. 2007. Molecular mechanisms underlying the probiotic effects of *Escherichia coli* Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. *Cellular Microbiology* 9: 804–816.

Chapter 5
Effects of Prebiotics and Probiotics
on the Host Immune Response

Michael H. Kogut and Christina L. Swaggerty

Abstract The gastrointestinal (GI) tract is the largest interface between an animal's internal milieu and its exterior environment. As such, it forms a physical barrier between the two environments. However, the function of the GI tract in the well-being of an animal is more complex than this passive role. The GI tract not only regulates the selective entry of nutrients while keeping vigilant against pathogens, it is largely responsible for shaping the immune response. Through specialized receptors and other general mechanisms, the GI tract senses changes in its environment and actively responds to the changes. These responses allow the intestine to contribute to the defense against microbes as well as control and regulate the local immune response. In addition, the luminal microbial ecosystem is a highly complex community of primarily bacterial microbes that communicates extensively with itself and the host. The microbial community has major influences on the host, including effects on nutrient absorption, cancer, inflammation, host metabolism, barrier function, and gut function (neuromotor, immunological, vascular) among others. Regulation of the immune response is the basis for the use of probiotics and prebiotics reviewed in this chapter.

Abbreviations

BSA   Bovine serum albumin
GALT   Gut-associated lymphoid tissue
GI   Gastrointestinal
Ig   Immunoglobulin

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IFN   Interferon
IL   Interleukin
LPS   Lipopolysaccharide
MAMP   Microbial-associated molecular pattern
NLR   NOD-like receptor
PRR   Pattern recognition receptor
SE   *Salmonella enterica* serovar Enteritidis
ST   *Salmonella enterica* serovar Typhimurium
TGF   Transforming growth factor
TLR   Toll-like receptor
TNF   Tumor necrosis factor
TT   Tetanus toxoid

5.1   Overview of the Immune Response

The immune system is a multifaceted arrangement of membranes (skin, epithelial, mucus), cells, and molecules whose function is to purge a host of invading pathogens and cancer cells. Working together, the various components of the immune system perform a careful balance of being lethal enough to kill pathogens or cancer cells yet sufficiently specific to not cause extensive damage to healthy "self" tissues of the host. A properly functioning immune system is a requirement for a "healthy" life in the modern animal world.

The foremost function of an immune response is to identify and eliminate pathogenic infections. The immune system of vertebrates is made up of two functional elements – innate and acquired responses – which contrast in their mechanisms of pathogen recognition (Medzhitov and Janeway 1997a). The innate system uses germ-line encoded receptors, known as pattern recognition receptors (PRRs) that recognize the evolutionarily conserved molecular components [microbial-associated molecular patterns (MAMPs)] of infectious microbes (Fearon and Locksley 1996; Medzhitov and Janeway 1997b; Medzhitov and Janeway 2000; Janeway and Medzhitov 2002; Carpenter and O'Neill 2007). The acquired response uses highly specific antigen receptors on T and B lymphocytes that are generated by random processes by gene rearrangement (Fearon and Locksley 1996; Carpenter and O'Neill 2007). Therefore, antigen receptors of the acquired immune system can be produced for any given antigen. One should not regard the acquired and innate systems as autonomous networks working independently of each other, however. Instead, the two systems are heterogeneous constituents of a single interdependent network.

Nowhere is this interdependence between the innate and acquired systems more pertinent than at the mucosal surface of the GI tract, which contains the largest number of immune cells and the highest concentration of pathogens and potential pathogens but also harmless dietary antigens and large populations of commensal bacterial flora (Neish 2009). Thus, the mucosal immune system must be tightly controlled to assess and respond to antigens to which it is exposed and mount an appropriate effector or regulatory response (Monteleone et al. 2006; Neish 2009). Hence, the

concurrent establishment of resident intestinal microbiota and the development of resident immune cells produces a state of "physiological inflammation" that is responsible for a rapid host response to a pathogenic infection (Sansonetti 2004).

5.2 Components of Gastrointestinal Mucosal Immunity

5.2.1 Innate Responses

The primary cell type that intervenes between the intestinal lumen community and the immune system is the epithelial cell. In fact, it may be the most significant part of the mucosal immune system. The intestinal epithelium is a critical component of a communications network that is essential for transmitting signals generated in response to infection with microbial pathogens to cells of the innate and acquired immune systems in the underlying intestinal mucosa (Winkler et al. 2007; Artis 2008). Intestinal epithelial cells are in a continuous state of response to the normal microbial ecology and, through their products, regulate the composition of this community. Because of these functions, the epithelium is considered a "microbial sensor" (Artis 2008). Recognition of structural components of microbes by membrane-associated and cytosolic PRRs of epithelial cells is a primary influence on the development of immune responses. Specifically, Toll-like receptors (TLRs) and the nucleotide-binding domain, leucine-rich repeat-containing family of proteins [nucleotide-binding oligomerization (NOD)-like receptors (NLRs)], are two important families of PRRs required for microbial recognition, gut homeostasis, and induction and regulation of the innate and adaptive immune responses (Kim et al. 2004; Rakoff-Nahoun et al. 2004). Recognizing components of microbes triggers both innate and adaptive immune responses that eliminate pathogens and shape the intestinal microflora, including the synthesis of antimicrobial peptides, proinflammatory cytokines and chemokines (Trinchieri and Sher 2007). Also triggered are the secondary anti-inflammatory responses required for the resolution of inflammation (He et al. 2007). In addition, a humoral component of the innate immune system has been identified, comprising natural antibodies (Matson et al. 2005). Natural antibodies are unique among immunoglobulin (Ig) molecules because their presence does not require previous exposure to a specific antigen. Natural antibodies act as recognition molecules capable of opsonizing invading pathogens and initiating the complement enzyme cascade (Carroll and Prodeus 1999).

5.2.2 Acquired Responses

Gut-associated lymphoid tissue (GALT) holds a crucial component of the total immunological capacity of the host in recognizing and selectively handling specific antigens for the initiation of acquired immune responses mediated by T and B

lymphocytes (Bauer et al. 2006). The GALT, including Peyer's patches, constitutes the largest mass of immune cells in the body and provides specific, acquired immune responses. Peyer's patches, located in the lamina propria and submucosa of the small intestine, are discrete areas of organized lymphoid tissue with defined T- and B-lymphocyte areas. Close, tightly orchestrated interactions between the intestinal epithelium and the GALT system are critical for normal intestinal absorptive and immunological functions.

5.2.3   Microbiota

As a whole, the intestinal microbiota can be considered as an organ within the host. The immune system continuously adapts to the intestinal microbiota in a dynamic cross-talk manner, where intestinal epithelial cells instruct noninflammatory responses for steady-state control of bacterial growth or triggering inflammatory mechanisms that can clear the GI tract of harmful invaders (Kelley et al. 2005; Corthesy et al. 2007; Winkler et al. 2007; Artis 2008; Abreu 2010). The system is complex and robust in the sense that many players with partially overlapping roles act to maintain the integrity of the intestinal mucosal barrier.

Microbes are continuously interacting with the host through direct interactions with epithelial and subepithelial components of the GALT both within the lumen and after translocation. They are also interacting indirectly through production of a variety of secreted factors that interact with both innate and adaptive components of the GALT. The response of the host to these microbial factors is represented by a normal state of immunological ignorance, tolerance, and immunity (both humoral and cellular) resulting from both microbial regulation of host immunity and host regulation of the microbial ecology. The relationship between the host and microbes is long term, with its onset early in life so the community of luminal microbes is immunologically perceived as "self" – as if it were an organ system unto itself that actively participates in the host's homeostasis (Kelley et al. 2005; Corthesy et al. 2007; Winkler et al. 2007; Artis 2008; Neish 2009; Abreu 2010).

5.3   Modulation of Intestinal Immunity

Close, tightly orchestrated interactions between the intestinal epithelium and the mucosa-associated immune system are critical for normal intestinal absorptive and immunological functions. Recent data indicate that the commensal intestinal microbiota represents a major modulator of intestinal homeostasis. Because there is a beneficial and symbiotic relationship between the host and the endogenous microbiota of the GI tract, strategies aimed at directly modulating the intestinal microbiota with regard to disease prevention or treatment can be developed.

5.3.1 Probiotics

One strategy for modulating intestinal immunity involves administering viable probiotic bacteria. Probiotics are defined as live, well-defined bacteria or yeasts that, when administered in adequate amounts, confer a health benefit on the host GI tract. Although this beneficial effect was originally thought to be due to improvements in the intestinal microbial balance, there is increasing evidence that the success of probiotics is associated with their potential to modulate barrier properties of the intestinal wall and host immunity. They accomplish this by allowing transient release of local and systemic acting bioactive compounds from the intestinal epithelium, including mucins, defensins, bacterocins, and cytokines and chemokines, which promote adaptive immune responses such as by secretory IgA and regulatory T cells (see recent reviews by Corthesy et al. 2007; Hord 2008; Neish 2009).

5.3.2 Prebiotics

Prebiotics are defined as dietary supplements that are nondigestible in the host but that provide a beneficial physiological effect on the host by selectively stimulating favorable growth or activity of a limited number of indigenous bacteria. Prebiotics function complementary to, and possibly synergistically with, probiotics (Hord 2008). Prebiotics are often polysaccharides that can withstand acidic and enzymatic digestion. They can be utilized by probiotics and gut microflora for growth and activities that benefit the host's health including enhancing immunity, especially increasing titers of secretory and serum immunoglobulins (Janardhana et al. 2009).

5.4 Effects of Probiotics and Prebiotics on Immunity in Poultry

Probiotics and prebiotics are becoming favorable alternatives to antibiotics as products that reduce the populations of food-borne pathogenic bacteria and eliminate pathogens that negatively impact animal production or food safety in the poultry industry. Over the last decade, an increasing number of studies have been conducted to evaluate the effects and mechanisms of probiotics on avian immunity.

5.4.1 Innate Immunity

The acute-phase response is an early innate response characterized by inflammation, fever, muscle catabolism, and anorexia. Using injected lipopolysaccharide (LPS) to

induce an acute-phase response in 14-day-old chickens, Jiang et al. 2010 evaluated whether dietary supplementation of a multispecies probiotic would alleviate growth suppression and anorexia associated with the acute phase response. Probiotic supplementation lessened the anorexic effects of LPS, resulting in improvement in body weight gain when compared to the non-LPS-injected control birds.

Haghighi et al. (2006) provided evidence that probiotic treatment of nonimmunized chickens increased induction of natural antibodies. Following application of a three-strain probiotic to 1-day-old chickens, serum and intestinal antibodies against tetanus toxoid (TT) and *Clostridium perfringens* α-toxin as well as intestinal IgA reactive to bovine serum albumin (BSA) were increased in nonimmunized chickens. In addition, IgG antibodies reactive to TT were increased in the intestines of probiotic-treated chickens when compared to untreated controls. In serum, IgG and IgM reactive to TT and α-toxin were increased in probiotic-treated, nonimmunized chickens compared to levels in untreated controls.

Recently, the possible role of antimicrobial peptides in probiotic-mediated protection against *Salmonella* in chickens was investigated (Akbari et al. 2008). Chickens given only probiotics had no change in the expression of any of the five avian antimicrobial peptide genes investigated. *Salmonella* infection led to elevated expression of all the antimicrobial genes studied; however, probiotic treatment prior to infection eliminated the effect of *Salmonella* infection on the expression of antimicrobial genes. The results imply that expression of antimicrobial peptides may be repressed by probiotics during a *Salmonella* infection or indicate that because there is dramatic reduction in *Salmonella* load in the intestine these genes may not be induced.

Probiotics have also been shown to have immune-modulating effects on cellular components of the innate response of chickens. Heterophils are the primary polymorphonuclear white blood cell of poultry. Heterophils isolated from chickens fed three separate probiotic bacterial isolates (*Bacillus subtilis*, *Lactococcus lactis*, or *Lactobacillus acidophilus*) were shown to have a significant increase in functional activities when compared to heterophils isolated from untreated control birds (Farnell et al. 2006). Likewise, intestinal leukocytes isolated from both layer-type and meat-type chickens fed probiotic lactobacilli had increased phagocytic and bactericidal activity against *Salmonella enterica* serovar Enteritidis (SE) (Koenen et al. 2004). Conversely, probiotic treatment of young chickens has no consistent effect on macrophage phagocytosis of SE (Higgins et al. 2007).

5.4.2 Acquired Immunity

There was significantly higher antibody production in chickens fed probiotics than in control birds (Kabir et al. 2004). In addition, these investigators found that the differences in the weights of the spleen and bursa of probiotic-fed and conventional-fed broilers could be attributed to different levels of antibody production in response to sheep red blood cells (Kabir et al. 2004). Similarly, in a group of broiler chickens

given a probiotic supplement the antibody titer was significantly higher at 5 and 10 days after immunization than in the controls (Khaksefidi and Ghoorchi 2006). Early colonization of intestines of 1-day-old chickens by a probiotic containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus faecalis* results in significant enhancement of the systemic IgM response to sheep red blood cells (Haghighi et al. 2005). Dalloul et al. (2003a) found that feeding chickens a *Lactobacillus*-based probiotic had a positive impact in that it stimulated some of the early immune responses against *Eimeria acervulina*, characterized by early interferon (IFN)-γ and interleukin (IL)-2 secretions, resulting in improved local immune defenses against coccidiosis. Numerous studies have demonstrated that giving probiotics has beneficial effects on humoral immune responses (Dalloul et al. 2003b; Huang et al. 2004; Koenen et al. 2004; Revolledo et al. 2006; Nayebpor et al. 2007; Mathivanan et al. 2007; Apata 2008).

Chicken cecal tonsil and splenic mononuclear cells have a differential response to structural constituents of *L. acidophilus* (Brisbin et al. 2008). Immune system genes were induced in cecal tonsil cells more rapidly than spleen cells when the cells were exposed to bacterial stimuli; the most potent stimulus for cecal tonsil cells was DNA, and for spenocytes it was bacterial cell wall components. The genes of the transcription factors STAT2 and STAT4 were highly induced in the splenocytes and cecal tonsil cells. Furthermore, the expression of IL-18, IFNα, and IFNγ genes were up-regulated in cecal tonsil cells after treatment with *L. acidophilus* DNA. This group further investigated the immunological mechanisms of probiotic treatment during infection with *Salmonella enterica* serovar Typhimurium (ST) (Haghighi et al. 2008). There was no significant difference in IL-6 or IL-10 gene expression in cecal tonsils of chickens given a probiotic or a conventional diet. Although ST infection resulted in a significant increase in IL-12 expression in cecal tonsils, chickens treated with probiotics prior to experimental infection with *Salmonella* had IL-12 expression levels similar to that observed in uninfected control chickens. Furthermore, treatment of birds with probiotics resulted in a significant decrease in IFNγ gene expression in cecal tonsils of chickens infected with *Salmonella* compared to the *Salmonella*-infected birds not treated with probiotics. Thus, repression of IL-12 and IFNγ expression in the GALT of the chicken is associated with probiotic-mediated reduction in intestinal colonization with ST.

Although the use of prebiotics as feed additives in poultry diets have been shown to improve feed conversion, reduce disease severity, and lower mortality, there have been few studies evaluating their effects on the immune response of poultry (Fukata et al. 1999; Chen et al. 2003). However, two recent studies have shown that prebiotics either alone (Janardhana et al. 2009) or in combination with a probiotic culture (Li et al. 2009) had beneficial effects on the local systemic immune responses, including serum antibody titers, T-cell numbers, and relative immune organ weights. It should be pointed out that two oligosaccharides fed to chickens caused a reduction in relative B-cell numbers and the mitogen responsiveness of T cells, and they had no effect on the expression of proinflammatory or antiinflammatory cytokine gene expression in cells isolated from cecal tonsils (Janardhana et al. 2009).

5.5 Effects of Probiotics and Prebiotics on Immunity in Swine

As in poultry, most of the studies on the effects of probiotics on intestinal and systemic immunity in swine have been reported during the last few years. In early studies using a probiotic *Enterococcus faecium* strain in sows and piglets, the total serum IgG of the sows was unaffected. Piglets of both groups showed similar IgG levels up to 5 weeks after birth, but by 8 weeks of age the total IgG levels of the probiotic-treated animals were significantly lower. No differences were observed in the populations of CD4+ (helper) and CD8+ (cytotoxic) T cells in Peyer's patches, although the number of CD8+ T cells in the jejunal epithelium of piglets of the probiotic-treated group were significantly reduced (Pollmann et al. 2005; Scharek et al. 2005; Taras et al. 2006). Changes in the humoral immune system were observed in swine (Scharek et al. 2007a), as were changes in the immune cell populations in the intestinal intraepithelial layer and the lamina propria (Scharek et al. 2007b). Szabo et al. (2009) investigated the influence of treating weanling pigs orally with the probiotic bacterium *Enterococcus faecium* to determine its effect on *Salmonella typhimurium* strain DT104 (ST DT104) infections. Interestingly, the probiotic treatment resulted in greater production of specific antibodies (serum IgG, IgM, and IgA) against ST, but it enhanced intestinal colonization by ST DT104. Administration of the probiotic *Enterococcus faecium* also modulated the composition of the blood lymphocyte populations in piglets (Duncker et al. 2006; Scharek et al. 2007b). On the other hand, the effect of the probiotic strain *Escherichia coli* Nissle 1917 had only minor effects on the distribution of mucosal immune cells in the gut of healthy young pigs (Duncker et al. 2006). In addition, mRNA analysis revealed no changes in mucosal mRNA expression of cytokines [IFNγ, tumor necrosis factor α (TNFα), transforming growth factor β (TGFβ), IL-10] or antimicrobial peptides (PR-39, NK-lysin, prepro-defensin-β1, protegrins) (Duncker et al. 2006). Administration of live yeast (*Saccharomyces cerevisiae* spp. *boulardii*) to weaned pigs for 3–4 weeks improved growth performance after weaning and increased the number of macrophages at various sites of the small intestine (Baum et al. 2002; Bontempo et al. 2006).

Changing immune parameters during pregnancy have previously been reported and have been suggested to contribute to increased susceptibility to infections. Thus, in a recent study, the effect of probiotics on the peripartum immune status of pregnant sows was studied (Schierack et al. 2009). Feed supplementation with a probiotic strain of *Bacillus cereus* was shown to reverse partially the immunological shifts due to pregnancy. The proliferative response of peripheral blood mononuclear cells of probiotic-treated sows increased during pregnancy. Bacterial antigens primarily stimulated the proliferation of naive CD21+ lymphocytes; and the relative CD21+ lymphocyte numbers were elevated in the probiotic-treated group in the absence of effects on other immune cell populations (Schierack et al. 2009).

One study has investigated the possible synergistic action of a prebiotic (fructooligosaccharides) with a probiotic strain of *Bifidobacterium animalis* on TLR gene expression in various organs of weaned piglets (Trevisi et al. 2008). The prebiotic or probiotic doses did not affect expression of the TLR2-encoding gene in the jejunum or the TLR4- and TNFα-encoding gene expressions in the jejunum, liver, or

ileocecal lymph nodes of the pigs 2 weeks after weaning. A synergistic, dose-dependent effect of *B. animalis* on the expression of the TLR2 gene in the lymph nodes was observed when fructooligosaccharides were added to the diet. TNFα-encoding gene expression was positively correlated with TLR4- and TLR2-encoding gene expression. Moreover, expression of the TLR4 showed a positive correlation with TLR2-encoding gene expression.

5.6 Effects of Probiotics and Prebiotics on Immunity in Cattle

Relatively few reports have been published describing the effects of probiotics on immune function in cattle. Thompson et al. (2009) reported on the transcriptional profile of selected innate immune genes in primary bovine intestinal epithelial cells that were assessed over a time course of incubation with the probiotic *Lactobacillus plantarum* (Lp299v). Incubation of bovine intestinal epithelial cells with Lp299v was performed in vitro, and gene expression was analyzed through a quantitative reverse transcriptase-polymerase chain reaction. Cytokine genes (IFNα, IFNβ, IL-6, TNFα) were up-regulated throughout the 12-h exposure. In addition, MyD88, a universal adapter protein used by TLRs to activate the transcription factor NF-κB, showed the most significant increase in gene expression at the 6- and 12-h time points. Supplementing the diet of neonatal calves with a prebiotic product was found to have virtually no effect on the number of CD4+ and CD8+ T lymphocytes in the peripheral blood for the 8 weeks of the trial (Heinrichs et al. 2009). Likewise, there were no effects on fecal IgA concentrations in the calves on the control diet when compared to those with the probiotic-supplemented diet observed during the experimental period.

References

Abreu, M.T. 2010. Toll-like receptor signaling in the intestinal epithelium: How bacterial recognition shapes intestinal function. *Nature Reviews Immunology* 10: 131–143.

Akbari, M.R., H.R. Haghighi, J.R. Chambers, et al. 2008. Expression of antimicrobial peptides in cecal tonsils of chickens treated with probiotics and infected with *Salmonella enterica* Serovar Typhimurium. *Clinical and Vaccine Immunology* 15: 1689–1693.

Apata, D.F. 2008. Growth performance, nutrient digestibility, and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. *Journal of the Science of Food and Agriculture* 88: 1253–1258.

Artis, D. 2008. Epithelial cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nature Reviews Immunology* 8: 413–420.

Bauer, E., B.A. Williams, H. Smidt, et al. 2006. Influence of the gastrointestinal microbiota on the development of the immune system in young animals. *Current Issues Intestinal Microbiology* 7: 35–52.

Baum, B., E.M. Liebler-Tenorio, M.L. Eriss, et al. 2002. *Saccharamyces boulardii* and *Bacillus cereus* var. *toyoi* influence the morphology and the mucins of the intestine of pigs. *Zeitschrift für Gastroenterologie* 40: 277–284.

Bontempo, V., A. Di Giancamillo, G. Savoini, et al. 2006. Live yeast dietary supplementation acts upon intestinal morpho-functional aspects and growth in weanling piglets. *Animal Feed Science and Technology* 129: 224–236.

Brisbin, J.T., H. Zhou, J. Gong, et al. 2008. Gene expression profiling of chicken lymphoid cells after treatment with Lactobacillus acidophilus cellular components. *Developmental and Comparative Immunology* 32: 563–574.

Carpenter, S., and L.A. O'Neill. 2007. How important are Toll-like receptors for antimicrobial responses? *Cellular Microbiology* 9: 1891–1901.

Carroll, M.C., and A.P. Prodeus. 1999. Linkages of innate and adaptive immunity. *Current Opinion in Immunology* 10: 36–40.

Chen, H.L., D.F. Li, B.Y. Chang, et al. 2003. Effects of Chinese herbal polysaccharides on the immunity and growth performance of young broilers. *Poultry Science* 82: 364–370.

Corthesy, B., H.R. Gaskins, and A. Mercenier. 2007. Cross-talk between probiotic bacteria and the host immune system. *Journal of Nutrition* 137: 781S–790S.

Dalloul, R.A., H.S. Lillehoj, T.A. Shellem, et al. 2003a. Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus*-based probiotic. *Poultry Science* 82: 62–66.

Dalloul, R.A., H.S. Lillehoj, T.A. Shellem, et al. 2003b. Intestinal immunmodulation by Vitamin A deficiency and Lactobacillus-based probiotic in *Eimeria acervulina*-infected broiler chickens. *Avian Diseases* 47: 1313–1320.

Duncker, S.C., A. Lorentz, B. Schroeder, et al. 2006. Effect of orally administered probiotic *E. coli* strain Nissle 1917 on intestinal mucosal immune cells of healthy young pigs. *Veterinary Immunology and Immunopathology* 111: 239–250.

Farnell, M.B., A.M. Donoghue, F.S. de los Santos, et al. 2006. Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. *Poultry Science* 85: 1900–1906.

Fearon, D.T., and R.M. Locksley. 1996. The instructive role of innate immunity in the acquired immune response. *Science* 272: 50–54.

Fukata, T., K. Sasai, T. Miyamoto, et al. 1999. Inhibitory effect sof competitive exclusion and fructooligosaccharide, singly and in combination, on *Salmonella* colonization in chicks. *Journal of Food Protection* 62: 229–233.

Haghighi, H.R., J. Gong, C.I. Gyles, et al. 2005. Modulation of antibody-mediated immune response by probiotics in chickens. *Clinical and Diagnostic Laboratory Immunology* 12: 1387–1392.

Haghighi, H.R., J. Gong, C.I. Gyles, et al. 2006. Probiotics stimulate production of natural antibodies in chickens. *Clinical and Vaccine Immunology* 13: 975–980.

Haghighi, H.R., M. Faizel, A. Careem, et al. 2008. Cytokine gene expression in chicken cecal tonsils following treatment with probiotics and *Salmonella* infection. *Veterinary Microbiology* 136: 225–233.

He, B., W. Xu, P.A. Santini, et al. 2007. Intestinal bacteria trigger T cell-independent immunoglobulin A$_2$ class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity* 26: 812–826.

Heinrichs, A.J., C.M. Jones, J.A. Elizondo-Salzar, et al. 2009. Effects of prebiotic supplement on health of neonatal dairy calves. *Livestock Science* 125: 149–154.

Higgins, S.E., G.F. Erf, J.P. Higgins, et al. 2007. Effect of probiotic treatment on intestinal macrophage numbers and phagocytosis of *Salmonella* Enteritidis by abdominal exudates cells. *Poultry Science* 86: 2315–2321.

Hord, N.G. 2008. Eukaryotic-microbiota crosstalk: Potential mechanisms for health benefits of probiotics and probiotics. *Annual Review of Nutrition* 28: 215–231.

Huang, M.K., Y.J. Choi, R. Houde, et al. 2004. Effects of lactobacilli and acidophilic fungus on the production performance and immune responses in broiler chickens. *Poultry Science* 83: 788–795.

Janardhana, V., M.M. Broadway, M.P. Bruce, et al. 2009. Prebiotics modulate immune responses in the gut-assoicated lymphoid tissue of chickens. *Journal of Nutrition* 139: 1404–1409.

Janeway, C.A., and R. Medzhitov. 2002. Innate immune recognition. *Annual Review of Immunology* 20: 1–28.

Jiang, Z., G. Schatzmayr, M. Mohno, et al. 2010. Net effect of acute phase response-partial alleviation with probiotic supplementation. *Poultry Science* 89: 28–33.

Kabir, S.M.L., M.M. Rahman, M.B. Rahman, et al. 2004. The dynamics of probiotics on growth performance and immune response in broilers. *International Journal of Poultry Science* 3: 361–364.

Kelley, D., S. Conway, and R. Aminov. 2005. Commensal gut bacteria: Mechanisms of immune modulation. *Trends in Microbiology* 26: 326–333.

Khaksefidi, M.A., and T. Ghoorchi. 2006. Effect of probiotix on performance and immunocompetence in broiler chicks. *International Journal of Poultry Science* 43: 296–300.

Kim, J.G., S.J. Lee, and M.F. Kagnoff. 2004. Nod1 is an essential signal transducer in intestinal epithelial cells infected with bacteria that avoid recognition by Toll-like receptors. *Infection and Immunity* 72: 1487–1495.

Koenen, M.E., J. Kramer, R. van der Hulst, et al. 2004. Immunmodulation by probiotic lactobacilli in layer and meat-type chickens. *British Poultry Science* 45: 355–366.

Li, S.P., X.J. Zhao, and J.Y. Wang. 2009. Synergy of *Astragalus* polysaccharides and probiotics (*Lactobaciilus* and *Bacillus cereus*) in immunity and intestinal microbiota in chicks. *Poultry Science* 88: 519–525.

Mathivanan, R., K. Kalaiarasi, and K. Panchagavya. 2007. *Andrographis paniculata* as alternative to antibiotic growth promoters on haematological, serum biochemical parameters and immune status of broilers. *International Journal of Poultry Science* 44: 198–204.

Matson, K.D., R.E. Rickelfs, and K. Klasing. 2005. A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental and Comparative Immunology* 29: 275–286.

Medzhitov, R., and C.A. Janeway Jr. 1997a. Innate immunity: The virtues of a nonclonal system of recognition. *Cell* 91: 295–298.

Medzhitov, R., and C.A. Janeway Jr. 1997b. Innate immunity: Impact of the adaptive immune response. *Current Opinion in Immunology* 9: 4–9.

Medzhitov, R., and C.A. Janeway Jr. 2000. Innate immune recognition: Mechanisms and pathways. *Immunological Reviews* 73: 89–97.

Monteleone, G., H. Peluso, D. Fina, et al. 2006. Bacteria and mucosal immunity. *Digestive and Liver Disease* 38(Suppl. 2): S256–S260.

Nayebpor, M., M. Farhomand, and A. Hashemi. 2007. Effects of different levels of direct fed microbial (*Primalac*) on growth performance and humoral immune response in broiler chickens. *Journal of Animal and Veterinary Advances* 6: 1308–1313.

Neish, A.S. 2009. Microbes in gastrointestinal health and disease. *Gastroenterology* 136: 65–80.

Pollmann, M., M. Nordoff, A. Pospischil, et al. 2005. Effects of a probiotic strain of *Enterococcus faecium* on the rate of natural Chamydia infection in swine. *Infection and Immunity* 73: 4346–4353.

Rakoff-Nahoun, S., J. Paglino, F. Eslami-Varzaneh, et al. 2004. Recognition of commensal microflora by Toll-like receptors for intestinal homeostasis. *Cell* 118: 229–241.

Revolledo, L., S.A. Ferriera, and A.J. Ferriera. 2006. Prevention of *Salmonella* Typhimurium colonization and organ invasion by combination treatment in broiler chicks. *Poultry Science* 88: 734–743.

Sansonetti, P.J. 2004. War and peace at mucosal surfaces. *Nature Reviews Immunology* 4: 953–964.

Scharek, L., J. Guth, K. Reiter, et al. 2005. Inlfuence of a probiotic *Enterococcus faecium* strain on development of the immune system of sows and piglets. *Veterinary Immunology and Immunopathology* 105: 151–161.

Scharek, L., B.J. Altherr, C. Tolke, et al. 2007a. Influence of the probiotic *Bacillus cereus* var toyoi on the intestinal immunity of piglets. *Veterinary Immunology and Immunopathology* 120: 136–147.

Scharek, L., J. Guth, M. Filter, et al. 2007b. Impact of the probiotic *Enterococcus faecium* NCIMB 10415 (SF68) and *Bacillus cereus* var. toyoi NCIMB 40112 on the development of serum IgG and fecal IgA of sows and their piglets. *Archives of Animal Science* 61: 223–234.

Schierack, P., M. Filter, L. Scharek, et al. 2009. Effects of *Bacillus cereus* var. toyoi on immune parameters of pregnant sows. *Veterinary Immunology and Immunopathology* 127: 26–37.

Szabo, I., L.H. Wieler, K. Tedin, et al. 2009. Influence of a probiotic strain of *Enterococcus faecium* on *Salmonella enterica* serovar Typhimurium DT104 infection in a porcine animal invasion model. *Applied and Environmental Microbiology* 75: 2621–2628.

Taras, D., W. Vahjen, M. Macha, et al. 2006. Performance, diarrhea incidence, and occurrence of *Escherichia coli* virulence genes during long-term administration of a probiotic *Enterococcus faecium* strain to sows and piglets. *Journal of Animal Science* 84: 608–617.

Thompson, A., E. Van Moorlehem, and P. Aich. 2009. Probiotic-induced priming of innate immunity to protect against rotaviral infection. *Probiotics and Antimicrobial Proteins*. doi:10.1007/s12602-009-9032-9.

Trevisi, P., S. De Flippo, L. Minieri, et al. 2008. Effect of fructo-oligosaccharides and different levels of *Bifidobacterium animalis* in a weaning diet on bacterial translocation and Toll-like receptor gene expression in pigs. *Nutrition* 24: 1023–1029.

Trinchieri, G., and A. Sher. 2007. Cooperation of Toll-like receptor signals in innate immune defense. *Nature Reviews Immunology* 7: 179–190.

Winkler, P., D. Ghadimi, J. Schrezenmeir, et al. 2007. Molecular and cellular basis of microflora–host interactions. *Journal of Nutrition* 137: 756S–772S.

Part II
Current and Future Status of Practical Applications and Challenges

Chapter 6
Current Status of Practical Applications: Pets

Brittany M. Vester Boler and George C. Fahey Jr.

Abstract Probiotics are marketed in the human food sector as being beneficial to gut health. As is often the case, this led to similar trends in the pet food and supplement markets. Although it is difficult to incorporate probiotics into pet diets because of processing problems, supplements and diets containing probiotics are now available. There is limited oversight of these products, however, so their application in a clinical setting is limited. Despite this situation, research on the topic is continuing to increase, and more outcomes regarding gut health are being evaluated. Overall, many of the probiotics evaluated in dogs and cats had positive effects on immune health, and some strains reduced potentially pathogenic bacterial species in the large bowel, all without affecting nutrient digestibility. There is more research needed, especially in regard to animals in diseased states, the determination of efficacious dosages for each bacterial strain, and evaluating the use of symbiotics.

6.1 Introduction

The companion animal industry continues its robust growth with a global market value of approximately US$60 billion for pet food and pet care products, with dog and cat food making up more than 70% of the market. Pet food sales have continued to rise in times of recession, growing 4.5% from 2008 to 2009, which translates to US$17.77 billion in sales (Packaged Facts 2010). This market is driven in part by an increase in pet ownership, humanization of pets by owners, and an increased popularity of commercially produced pet foods (Higgins 2007). As more people treat

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their pets as a part of the family, it has led to a growing market of pet foods and supplements with proven health benefits as are marketed to humans. Many people also expect functional ingredients with noted benefits to be included in pet diets or supplements.

Probiotics have the potential to play a major role in the development of new pet food products now and in the future based on several factors. Many owners are particularly concerned for their pet's health, which drives the demand for high-quality foods, especially those containing functional ingredients already marketed to humans. Probiotics have been reported to affect a number of biomarkers of health status in humans and animal models with minimal side effects, thereby making them ideal functional ingredients to address health concerns in pets. This is helped by the fact that probiotic-containing products also are being advertised for human consumption and so are recognized by people who purchase these foods and supplements for their animals. Additionally, more niche diets are being formulated to appeal to consumers with demands for high-quality, human-grade ingredients. Again, probiotics will be viewed as important components of these health-enhancing diets and supplements.

It is the intent of this chapter to provide a comprehensive review of the research that has been conducted to date in the dog and cat related to the application of probiotics. Several outcome variables have been measured to test the efficacy of these compounds in pet animals; but relative to the research reported on rodents, humans, livestock, and poultry, it is clear that much less research is available on this topic for pets than for many other animal species.

6.1.1 Application of Probiotics in the Pet Industry

Many probiotic studies using dogs or cats reported only the ability of the probiotic to survive in the gastrointestinal tract due to probiotic usage in pets being a relatively new concept. Many of these studies were performed prospectively to determine potential effects of certain bacterial strains. Little information is available as to the dosage that is most appropriate or efficacious for each bacterial species. A difficulty with creating pet foods that contain probiotic strains is the fact that most pet foods marketed today are in kibble or moist (canned) form. To create a kibble, ingredients are extruded, which uses high heat and pressure for short periods of time to cook the starch in the food. All canned moist diets undergo retort, which uses high heat to sterilize the product. Both processes (extrusion and retort) kill most bacteria in the food and would kill any probiotic strains. Therefore, any probiotic must be added after extrusion for the bacteria to survive; however, additions to diets after canning are not possible. A further hindrance of adding probiotics is that most pet foods have a guaranteed shelf life of up to 1 year. Many probiotics may not be able to survive this length of time in large enough numbers to remain efficacious; and therefore no label guarantee can be made. Owing to these limitations, probiotics often are included as supplements and not within the food itself.

Although some pet foods on the market are claimed to contain probiotics, the ability of companies to produce these diets that are shelf-stable is suspect. Weese and Arroyo (2003) evaluated 19 commercial pet foods claiming to contain probiotics (13 for dogs, 6 for cats). All diets were purchased from pet food retailers and tested prior to the indicated expiration date. None of the diets tested contained all of the organisms listed on the ingredient label. Of the 19 diets, 10 (53%) contained at least one microorganism listed on the ingredient label when tested; 5 (26%) products had no probiotic bacteria present. The foods tested contained between 0 and 1.8×10^5 colony forming units (cfu)/g, but it is unknown if this was the intended dose or due to decreases during storage. Furthermore, some diets listed bacterial fermentation products on the label without the bacteria themselves listed as ingredients, yet still claimed to contain a probiotic (Weese and Arroyo 2003).

Testing supplements was similar to the results of testing pet diets. The label claim was tested on 13 probiotic supplements (5 for human use, 8 for veterinary use) (Weese 2002). Few of these supplements, especially those marketed for veterinary use (3/8), provided exact bacterial species included in the supplement or the concentration present. Furthermore, all the veterinary products contained less than 2% of the listed concentration of bacteria on the label, with the highest actual concentration measured at 1.6×10^8 cfu. The author suggested that the dosage was below those known to elicit a response in humans, 1×10^9 to 1×10^{10} cfu (Weese 2002), and was also below the dosage used in much of the published literature regarding dogs and cats. Further testing of 44 human and veterinary product labels indicated striking issues with supplement label claim oversight (Weese 2003). Many of the products, intended for both human and veterinary use, contained misspelled (18%), misidentified, or nonexistent bacterial species (35%); and none stated the number of organisms that should be present at the expiration date. This clearly indicates that more needs to be done regarding proper ingredient labeling, oversight of claims, and guidelines for probiotic inclusion in supplements and pet foods.

6.1.2 Probiotic Evaluation in Vitro

Probiotic evaluation in vitro is leading to better understanding of the potential of many probiotic strains. To date, much of the research has focused on isolating potentially probiotic strains and evaluating their ability to survive in the upper gastrointestinal (GI) tract as well as their mucus adhesion capabilities. The ability of a probiotic to survive in the gut often is measured in vitro through tests of bile acid tolerance and pH tolerance. Mucus adhesion often is determined as a measure of the potential of a probiotic to attach and colonize within the GI tract. Attachment is important because bacteria that adhere to the mucus are in close contact to immune cells and therefore may be able to modulate the immune system and have antimicrobial activity toward potentially pathogenic bacterial species.

Lactic acid bacteria have been the most commonly studied probiotics in vitro for applications to pets. Several strains of lactobacilli [*L. rhamnosus* (human commercial

strain), *L. johnsonii* (human commercial strain)*, L. casei* (human commercial strain), *L. pentosus* (UK1A, isolated from dog feces), and *L. pentosus* (SK2A, isolated from dog jejunal chyme)], *Bifidobacterium lactis* Bb12 (human commercial strain), and *Enterococcus faecium* (animal commercial strains) (Rinkinen et al. 2000) were evaluated in vitro using dog jejunal chyme as inoculum. *L. rhamnosus* adhered to canine mucus better than all other bacterial strains. Pretreatment with jejunal chyme to simulate digestion limited adherence of all bacterial species, but three of the human-origin bacterial species, *L. johnsonii* (0% change), *L. casei* (0% change), and *Bifidobacterium lactis* (~53% decrease), were able to maintain more adhesion than other human strains and all strains isolated from dogs.

Other studies have evaluated isolated bacterial strains of canine origin regarding their ability to survive and attach to the mucus in the GI tract. Strompfova et al. (2006) evaluated canine-derived *L. fermentum* in vitro and in vivo. It was noted that 86% of the probiotic survived at pH 3 after 3 h, and 75.4% survived in the presence of bile in vitro. Approximately 2% adhesion to canine mucus was achieved and 2.7% to human mucus. In vivo, it was noted that fecal enterococci and lactobacilli species increased, but no changes were noted for *Escherichia coli* or *Staphylococcus* spp. after 7 days of supplementation. *Lactobacilli* spp. appear to have variable ability to survive in bile salts. Strains of *L. acidophilus* had higher bile tolerance than *L. reuteri* isolated from canine feces (McCoy and Gilliland 2007). There were two *L. reuteri* strains (X-27 and X-18), however, that were able to tolerate bile salts, inhibit *Salmonella typhimurium*, and produced reuterin, an antimicrobial substance. The adhesion capabilities of these strains, however, were not determined. Canine-derived *L. murinus* strains (LbP2, LbP6, LbP10) were able to withstand pH 3.5 (50% reduction for the most tolerant strain) and bile salt concentration of 0.3% (27% reduction for the most tolerant strain); they also had antimicrobial activity against *E. coli* and *Clostridium perfringens* strains (inhibitions zones between 10 and 17) and were able to adhere to mucus (5–16% adhesion) (Perelmuter et al. 2008).

Enterococcus strains of canine origin (six strains) have been evaluated as potential probiotics (Strompfová et al. 2004). Approximately 72–98% of the total population of selected strains were able to survive 1% bile salts, and 76–87% were able to survive pH 3. Percentage adherence to canine mucus ranged from 4% to 11%. Most strains (75%) produced bacteriocin-like inhibitory substances against select gram-positive bacteria. The question of safety regarding *Enterococcus faecium* probiotics has been raised (Rinkinen et al. 2003). *Enterococcus faecium* strains M74 and SF273 enhanced adhesion of *C. jejuni* 135% and 206%, respectively. If this also occurs in vivo, dogs could be considered carriers of C. *jejuni*, which can cause infections in humans.

In addition to the ability of probiotics to survive in the GI tract, some researchers have begun evaluating their ability to address health issues of dogs and cats. Duodenal samples from dogs with intestinal inflammation due to chronic enteropathies were used to evaluate the ability of a probiotic to decrease inflammation (Sauter et al. 2005). A probiotic cocktail [*L. acidophilus* (NCC2628 and NCC2766) and *L. johnsonii* (NCC2767)] decreased mRNA expression of anti-inflammatory cytokine interleukin-10 (IL-10) and thereby decreased the ratio of pro-inflammatory

cytokines [tumor necrosis factor α (TNFα), interferon γ (IFNγ), and IL-12 p40] to anti-inflammatory cytokine IL-10. Urinary oxalate stone formation is a common clinical problem in dogs and cats, and there is no current method of dissolving the stones. Therefore, several bifidobacteria and lactobacilli were evaluated for their ability to degrade ammonium oxalate (Murphy et al. 2009). None of the bifidobacteria was capable, but some select strains of lactobacilli were able to degrade it. *L. animalis* (27–68% reduction compared to control) and *L. murinus* (41–72% reduction compared to control) had oxalate degradation capability compared to the control.

These in vitro studies provide preliminary evidence that many probiotic strains may have beneficial effects in dogs and cats. The ability of these strains to withstand bile salts and pH and adhere to canine mucus indicate that they would survive the upper GI tract of the animal and survive to colonize the large bowel. Furthermore, these studies indicate that the use of probiotics in disease states may have beneficial effects on health biomarkers.

6.1.3   Probiotic Use in Vivo

Probiotic use in pets was reviewed by Vester and Fahey (2009); the review presented new literature and summarized previously reviewed work. The most common microbial species evaluated and utilized as probiotics in pets include *Lactobacillus acidophilus* and *Entercoccus faecium*. Although there are a moderate number of studies to date evaluating probiotic use in dogs, only three studies have currently been conducted in cats. Recent studies (2009 to the present) and research not reviewed by Vester and Fahey (2009) are described in detail in Table 6.1.

Probiotic use appears to have beneficial effects in vivo, but some conflicting results occur. It is difficult to compare results among studies because of the varying doses and modes of administration of the probiotic. Overall, it appears that probiotic bacteria, administered at a sufficiently high dose, lead to increases in gut probiotic bacterial species; and many exert antimicrobial effects leading to reduced numbers of potentially pathogenic bacterial species. During feeding of a probiotic, all studies that measured the probiotic in feces indicated the presence of, or a significant increase in, the probiotic species in the feces. Five studies indicated a decrease in fecal *C. perfringens* or *E. coli*, which often are considered pathogenic when allowed to grow above normal levels. One recent study noted an average 6% decrease in total fecal clostridial counts at 5 and 6 weeks after *Bifidobacteria animalis* AHC7 supplementation of adult dogs (O'Mahony et al. 2009). Furthermore, *Clostridium difficile* counts were 28% lower by week 6 in supplemented versus placebo-fed dogs.

One major issue with probiotic supplementation is that upon cessation of consumption the probiotic bacteria disappear. This indicates that probiotic bacteria likely are not attaching and colonizing the GI tract in great enough numbers to

Table 6.1 In vivo experiments, listed in chronological order, reporting effects of probiotics in cats and dogs

Reference	Outcome variables quantified	Animals/treatment (age, initial BW)	Dietary information; time on treatment	Daily probiotic dose; source	Major findings
O'Mahony et al. (2009)	Bacterial enumeration	11 Male and female dogs (average age 8.4 year)	Eukanuba premium performance; Time on treatment: Baseline 6 week; Treatment 6 week	1.5×10^9 cfu *Bifidobacterium animalis* AHC7/day	↓ Total fecal clostridia counts at wk 5 (7%) and 6 (5%)**; ↓ *C. difficile* counts (28%)**, but not *C. perfringens*, by week 6
Manninen et al. (2006)	Bacterial enumeration (DGGE)	5 Fistulated male and female beagles (aged 4–8 year)	Dry commercial diet Chemical composition: 23.0% CP; 13.0% Fat; 2.5% CF; 2.5% Ash; 92.0% DM; Diet ingredients: Cereal, Ceat, Animal byproducts, Fats, Fish and fish derivatives, Yeast; Time on treatment: 10 days	Between 1.4×10^7 and 5.9×10^7 cfu/day of LAB mixture 2 times/day; LAB mixture: 5.8×10^7 cfu/ml; LAB8 – *L. fermentum* 3.6×10^7 cfu/ml; LAB9 – *L. salivarius* 7.0×10^6 cfu/ml; LAB10 – *W. confuse* 8.0×10^6 cfu/ml; LAB11 – *L. rhamnosus* 3.9×10^7 cfu/ml; LAB12 – *L. mucosae*	LAB strains survived upper GI tract; Supplemented LAB strains displaced endogenous LAB strains during feeding; All LAB strains disappeared within 7 day of feeding cessation

Simpson et al. (2009)	Giardial cyst shedding Fecal antigen shedding Fecal IgA concentration Leukocyte phagocytic activity	20 Adult dogs Naturally acquired subclinical giardiasis	Purina dog chow (dry kibble diet) Time on treatment: 6 week	5×10^8 cfu E. faecium SF68/day Powdered form	No differences between treatments: Giardial cyst shedding Fecal antigen shedding Fecal IgA concentrations Leukocyte phagocytic activity
Marsella (2009)	Clinical signs of atopic dermatitis Blood and skin allergen-specific IgE	2 Adult beagles (breeding pair) with severe atopic dermatitis 16 Puppies (2 litters from the breeding pair) Litter 1 – no probiotic Litter 2 – probiotic Exposed to D. Farinae to induce atopic dermatosis	Adult diet: Science Diet Canine Adult Maintenance Puppy diet: Science Diet Puppy Healthy Development Savory Chicken Entrée dog food Time on treatment: Bitch – from wk 3 of gestation and throughout lactation Puppies – treatment started at 3 week of age until 6 month of age	L. rhamnosus GG capsules contained minimum of 20×10^9 cfu Bitch: fed 10 capsules/day Puppies: fed 5 capsules/day	↓ IgE titers against D. farinae by 24 week (~1,750 untreated litter vs. ~200 treated litter) ↓ Score for skin reactions to subjective intradermal injection of D. farinae at 1:25,000 dilution (score of 3 in untreated litter, score of 1 in treated litter)
Lappin et al. (2009)	FHV-1 shedding episodes FHV-1 DNA copies shed	12 Mixed sex cats (aged 1 year) Conjunctivitis and FHV-1 infected cats	Dry cat food Time on treatment: 112 day	5×10^8 CFU E. faecium SF68 /day	No change in lymphoblast transformation to concanavalin A No differences in lymphocyte responses to FHV-1 antigens ↓ Fecal microbial diversity in placebo fed cats **

(continued)

Table 6.1 (continued)

Reference	Outcome variables quantified	Animals/treatment (age, initial BW)	Dietary information; time on treatment	Daily probiotic dose; source	Major findings
Herstad et al. (2010)	Stool quality Days to normal stools Days to last vomiting episode	36 Adult dogs (age 4.1±3.3 year) with acute canine gastroenteritis N=21 placebo group N=15 probiotic group	Client-owned animals, consumed diet of owners choice	ZooLac Propaste (Chem Vet A/S Denmark) 2.85 billion live strains/ml: *L. farciminis* (porcine origin) *Pediococcus acidilactici* (unknown origin) *Bacillus subtilis* (soil origin) *Bacillus licheniformis* (soil origin) 1.35 billion thermo-stabilized/ml: *L. acidophilus* MA 64/4E (human origin) Provided based on BW: 1–10 kg=1 ml 10–25 kg=2 ml 25–50 kg=3 ml Administered 3 times/day Double dose administered until normalization of stools	↓ Days to normal stools in probiotic (1.3 day) vs. placebo (2.2 day) fed dogs**

BW body weight, *CF* crude fiber, *CFU* colony forming units, *CP* crude protein, *d day*, *DGGE* denaturing gradient gel electrophoresis, *DM* dry matter, *DNA* deoxyribonucleic acid, *FHV* feline herpes virus, *IgA* immunoglobulin A, *IgE* immunoglobulin E, *LAB* lactic acid bacteria, *d* days, *mo* month, *wk* week, *yr* year

**$P<0.05$

encourage a self-sustaining population. Biourge et al. (1998) indicated no detection of probiotic species (*Bacillus* CIP 5832) after 3 days of probiotic cessation, and Weese and Anderson (2002) noted *Lactobacillus rhamnosus* probiotic present in only one dog 72 h after cessation. Manninen et al. (2006) noted no *L. fermentum* (LAB8) or *L. mucosae* (LAB12) within 7 days of probiotic cessation. These results were contrary to those of Marciňáková et al. (2006), who noted survival of *E. faecium* EE3 after a 3-month cessation of probiotic treatment. The authors indicated that *E. faecium* EE3 is a strain that has adhesive capability in human and canine mucus (human 7.3% adhesion, canine 7.4%) (Marciňáková et al. 2006). Additionally Strompfova et al. (2006) noted the presence of the probiotic bacterium *L. fermentum* 6 months after supplement cessation, indicating that this bacterial species may be able to colonize the large bowel effectively.

More recent published literature, however, supports the findings of Biourge et al. (1998), Weese and Anderson (2002), and Manninen et al. (2006), in which rats were fed probiotics of canine origin (O'Mahony et al. 2009). After 5 days' cessation of feeding probiotic strains of *L. murinus/ruminus*, *Bifidobacterium globosum/pseudolongum*, or *B. animalis*, no probiotic bacteria were found in the feces. It should be noted that all animals in these studies were fed the respective probiotic for a similar number of days before cessation (approximately 7 days), and longer-term supplementation may lead to different results.

Total tract apparent macronutrient digestibility does not appear to be influenced by probiotic supplementation. Crude fiber digestion decreased 16% in mongrel puppies supplemented with *L. acidophilus* (Pasupathy et al. 2001), but the authors argued that this decrease in fiber utilization was negligible. Dry matter and CP digestibility tended to increase (2%) in adult dogs supplemented with *L. acidophilus* for 28 days (Swanson et al. 2002). This tendency, however, was noted in only one of two replicated experiments. Other studies evaluating digestibility found no differences due to probiotic supplementation. More recent studies have not reported nutrient digestibility data.

Studies evaluating the effects of probiotics supplementation on immunological changes or as disease treatments are limited but have increased in recent years. Benyacoub et al. (2003) noted an increase in fecal and plasma immunoglobulin A (IgA) in puppies fed an *Enterococcus faecium* strain for 44 weeks. An increased response to canine distemper virus – determined as an increased proportion of mature B cells and increased surface expression of major histocompatibility complex II (MHC II) molecule in monocytes – also was observed. This immune response would be beneficial because of the stressful time period of weaning in all species. In healthy adult dogs, increased serum IgG, decreased erythrocyte fragility, and increased white blood cell (WBC) and monocyte numbers were noted after 4 weeks of *L. acidophilus* supplementation (Baillon et al. 2004). Increased CD4+ lymphocyte concentration, but no changes in IgG, IgA, WBC counts, or response to vaccination were noted in kittens fed *E. faecium* for 20 weeks (Vier et al. 2007).

The most recent work evaluating probiotics has focused on disease states or immunocompromised dogs, cats, or animal model species. These studies provide critical information to practicing clinicians regarding the use of probiotics for treatment or prevention of common diseases of dogs and cats. Overall, it appears that

some diseases or acute conditions can be modified by probiotic supplementation; however, a preventive versus a treatment role has not been determined for all conditions studied. Previously reviewed literature noted mixed results regarding effects of probiotic supplementation of dogs with GI disease. Dogs with nonspecific dietary sensitivities had improved defecation frequency and fecal scores when supplemented with *L. acidophilus* (Pascher et al. 2008). Dogs with food-responsive diarrhea fed an elimination diet tended to have reduced duodenal IL-10 (proinflammatory cytokine) mRNA concentrations when supplemented with a lactobacilli cocktail, but these changes were not associated with improved clinical signs (Sauter et al. 2006).

Puppies predisposed to atopic dermatitis challenged with *Dermatophagoides farinae* and supplemented with *L. rhamnosus* GG from 3 weeks to 6 months of age had decreased IgE titers against *D. farinae* and a milder reaction to skin testing (Marsella 2009). Additionally, the interval from initiation of treatment to the first normal stool was shorter (1.3 vs. 2.2 days) in dogs suffering from acute gastroenteritis (defined as acute diarrhea or acute diarrhea and vomiting) when supplemented with a probiotic cocktail (*L. acidophilus*, *Pediococcus acidilactici*, *Bacillus subtillus*, *Bacillus licheniformis*, and *L. farciminis*) (Herstad et al. 2010). This indicates that probiotics may have a place in a clinical setting for acute treatment of gastroenteritis. Lastly, *Bifidobacterium animalis* and *L. murinus/ruminus* (AHC3133) of canine origin inhibited translocation of *Salmonella typhimurium* in challenged mice compared to placebo-treated mice (O'Mahony et al. 2009). The probiotic was provided prior to challenging with *Salmonella typhimurium*, indicating that the probiotic may provide a protective effect to bacterial infection.

Hasegawa et al. (1993, 1996) and Kanasugi et al. (1996) evaluated the use of heat-killed *Enterococcus faecalis* FK-23 oral supplementation to treat neutropenia in adult dogs. Neutropenia is a common side effect of cancer treatment drugs and is defined as a low level of circulating neutrophils. Supplemented dogs with artificially induced neutropenia had improved neutrophil-reconstituting capacity and an increased myeloid/erythroid ratio in the bone marrow. There may be a role for heat-killed *E. faecalis* in a clinical setting in dogs undergoing cancer treatment, especially considering that the availability of providing cancer treatment continues to increase and the fact that more people are considering it as a treatment option.

Not all studies, however, have indicated positive results from probiotic supplementation during disease. Dogs with naturally occurring giardiasis had no changes in cyst shedding, fecal IgA concentrations, or leukocyte phagocytic activity when supplemented with *E. faecium* SF68 for 6 weeks (Simpson et al. 2009). *E. faecium* SF68 supplementation (112 days) also had no effect on feline herpesvirus-1 (FHV-1) shedding or immune indices in cats with naturally occurring chronic FHV-1 infection (Lappin et al. 2009). There was, however, a trend for supplemented cats to have fewer days of conjunctivitis compared to placebo cats (16.8% vs. 30.9%, respectively). This may indicate a clinical effect but not an effect of underlying immune indices. Although this area of research is rapidly expanding, more clear trends are necessary to make specific recommendations. It is clear, however, that

probiotic supplementation appears to have a positive influence on the gut health of dogs and cats and may play a role in prevention and treatment of some diseases. Finding optimal doses as well as combinations of prebiotics and probiotics may provide further answers in this area.

6.1.4   Synbiotics in Dogs and Cats

Synbiotics are defined as the combination of probiotics and prebiotics to obtain a synergistic growth effect. This is not a novel concept, but information is limited in regard to canine and feline nutrition. Prebiotic supplementation is better studied than probiotic use in dogs and cats, but little has been done with a combination of the two. Three lactobacilli strains (*L. mucosae*, *L. acidophilus*, *L. reuteri*) were evaluated for their ability to have synergistic effects when grown on various carbohydrates (Tzortzis et al. 2004a). Measures of antagonistic compounds against *E. coli* and *Salmonella enterica* (serotype Typhimurium) were studied. The carbohydrate substrate used in conjunction with the lactobacilli species had an influence on the production of antibiotic compounds; however, each bacterial species reacted differently with each carbohydrate source. Each of the lactobacilli strains produced antimicrobial compounds when grown in sugar mixtures consisting of α-glucosidases (dp 1–4), this production was dose-responsive and more reactive at a lower pH (Tzortzis et al. 2004a).

Further work pairing the bacteria with an oligosaccharide synthesized by the bacterial species (*L. reuteri*) led to the most beneficial changes in microbial ecology in vitro using adult dog fecal inoculum (Tzortzis et al. 2004b). Combination of a galactosyl melibiose mixture (GMM)+*L. reuteri* increased beneficial bacterial populations and decreased clostridial and *E. coli* populations. Although the changes were not always more extensive than with a prebiotic alone, higher bifidobacteria and lactobacilli counts and greater acetate production were noted with the GMM+*L. reuteri* synbiotic (Tzortzis et al. 2004b). These in vitro studies may provide starting points for testing synbiotics in dogs and cats.

Only one study has evaluated synbiotic usage in dogs, and no studies in cats have been reported. In the dog study, replicate experiments were conducted. Dogs were randomly assigned to one of four dietary treatments: control, short-chain fructooligosaccharides (scFOS) alone, *Lactobacillus acidophilus* (1×10^9 cfu/day) alone, or 2 g scFOS+*L. acidophilus* (1×10^9 cfu/day) (Swanson et al. 2002). In one of the replicate experiments, fecal putrefactive compounds (biogenic amines, BCFA, phenols, indoles) decreased in dogs given the scFOS+*L. acidophilus* treatment. Because it occurred in only one of two replicate experiments, further work is needed to determine if this combination of prebiotic and probiotic has a beneficial synergistic effect in vivo. More evaluation of synbiotics in vivo is needed to determine potential synergistic effects and efficacious combinations in companion animals.

6.2   Conclusions

Overall, it is apparent that probiotics can be beneficial to companion animals. Although their use is still limited outside of research, the market for them is growing. Because of the difficulty of including these bacterial species in foods, it is likely that they will continue to be most commonly provided as supplements. Research indicates that probiotics as part of the daily consumption by companion animals leads to increases in fecal concentrations of that probiotic bacterium (indicating it reached the large bowel), reductions in potentially pathogenic bacteria, and stimulation of the immune system, with no change in nutrient digestibility. Despite this, it is still unclear what dosage is efficacious for each bacterial species and which bacterial species may be most beneficial. Furthermore, the use of synbiotics is limited but may lead to improvements in adhesion and colonization of the probiotic bacteria in the large bowel. Also, testing probiotics and synbiotics in more disease states is warranted, as is further research specific to cats. This is an area of research that is growing rapidly and will likely lead to advancements in the near future.

References

Baillon, M.A., Z.V. Marshall-Jones, and R.F. Butterwick. 2004. Effects of probiotic *Lactobacillus acidophilus* strain DSM13241 in healthy adult dogs. *American Journal of Veterinary Research* 65: 338–343.

Benyacoub, J., G.L. Czarnecki-Maulden, C. Cavadini, et al. 2003. Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune functions in young dogs. *Journal of Nutrition* 133: 1158–1162.

Biourge, V., C. Vallet, A. Levesque, et al. 1998. The use of probiotics in the diet of dogs. *Journal of Nutrition* 128: 2730S–2732S.

Hasegawa, T., H. Kanasugi, M. Hidaka, et al. 1993. Effect of oral administration of heat-killed *Enterococcus faecalis* FK-23 on the leukocyte-reconstituting capacity in immunosuppressed dogs. *Annals of the New York Academy of Sciences* 685: 369–371.

Hasegawa, T., H. Kanasugi, M. Hidaka, et al. 1996. Effect of orally administered heat-killed *Enterococcus faecalis* FK-23 preparation on neutropenia in dogs treated with cyclophosphamide. *International Journal of Immunopharmacology* 18: 103–112.

Herstad, H.K., B.B. Nesheim, T. L'Abee-Lund, et al. 2010. Effects of a probiotic intervention in acute canine gastroenteritis – A controlled clinical trial. *The Journal of Small Animal Practice* 51: 34–38.

Higgins, E. 2007. Global sales trends. In *Proceedings. 2007 Petfood Forum, 176–185*. Mt Morris: Watt Publishing Co.

Kanasugi, H., T. Hasegawa, T. Yamamoto, et al. 1996. Optimal dose of enterococcal preparation (FK-23) supplemented perorally for stimulation of leukocyte reconstitution in dogs treated with cyclophosphamide. *The Journal of Veterinary Medical Science* 58: 563–565.

Lappin, M.R., J.K. Veir, E. Satyaraj, et al. 2009. Pilot study to evaluate the effect of oral supplementation of *Enterococcus faecium* SF68 on cats with latent feline herpesvirus 1. *Journal of Feline Medicine and Surgery* 11: 650–654.

Manninen, T.J.K., M.L. Rinkinen, S.S. Beasley, et al. 2006. Alteration of the canine small-intestinal lactic acid bacterium microbiota by feeding of potential probiotics. *Applied and Environmental Microbiology* 72: 6539–6543.

Marciňáková, M., M. Simonavá, V. Strompfová, et al. 2006. Oral application of *Enterococcus faecium* strain EE3 in healthy dogs. *Folia Microbiologica* 51: 239–242.

Marsella, R. 2009. Evaluation of *Lactobacillus rhamnosus* strain GG for the prevention of atopic dermatitis in dogs. *American Journal of Veterinary Research* 70: 735–740.

McCoy, S., and S.E. Gilliland. 2007. Isolation and characterization of *Lactobacillus* species having potential for use as probiotic cultures for dogs. *Journal of Food Science* 72: M94–M97.

Murphy, C., S. Murphy, F. O'Brien, et al. 2009. Metabolic activity of probiotics – Oxalate degradation. *Veterinary Microbiology* 136: 100–107.

O'Mahony, D., K. Barry Murphy, J. MacSharry, et al. 2009. Portrait of a canine probiotic *Bifidobacterium* – From gut to gut. *Veterinary Microbiology* 139: 106–112.

Packaged Facts. 2010. U.S. Pet Market Outlook 2010–2011: Tapping into Post-Recession pet parent pending. 1–136.

Pascher, M., P. Hellweg, A. Khol-Parisini, et al. 2008. Effects of a probiotic *Lactobacillus acidophilus* strain on feed tolerance in dogs with non-specific dietary sensitivity. *Archives of Animal Nutrition* 62: 107–116.

Pasupathy, K., A. Sahoo, and N.N. Pathak. 2001. Effect of lactobacillus supplementation on growth and nutrient utilization in mongrel pups. *Archives of Animal Nutrition* 55: 243–253.

Perelmuter, K., M. Fraga, and Z. Pablo. 2008. *In vitro* activity of potential probiotic *Lactobacillus murinus* isolated from the dog. *Journal of Applied Microbiology* 104: 1718–1725.

Rinkinen, M., J. Matto, S. Salminen, et al. 2000. *In vitro* adhesion of lactic acid bacteria to canine small intestinal mucus. *Journal of Animal Physiology and Animal Nutrition* 84: 43–47.

Rinkinen, M., K. Jalava, E. Westermarck, et al. 2003. Interaction between probiotic lactic acid bacteria and canine enteric pathogens: A risk factor for intestinal *Enterococcus faecium* colonization? *Veterinary Microbiology* 92: 111–119.

Sauter, S.N., K. Allenspach, F. Gaschen, et al. 2005. Cytokine expression in an ex vivo culture system of duodenal samples from dogs with chronic enteropathies: Modulation by probiotic bacteria. *Domestic Animal Endocinology* 29: 605–622.

Sauter, S.N., J. Benyacoub, K. Allenspach, et al. 2006. Effects of probiotic bacteria in dogs with food responsive diarrhea treated with an elimination diet. *Journal of Animal Physiology and Animal Nutrition* 90: 269–277.

Simpson, K.W., M. Rishniw, M. Bellosa, et al. 2009. Influence of *Enterococcus faecium* SF68 probiotic on giardiasis in dogs. *Journal of Veterinary Internal Medicine* 23: 476–481.

Strompfová, V., A. Lauková, and A.C. Ouwehand. 2004. Selection of entercocci for potential canine probiotic additives. *Veterinary Microbiology* 100: 107–114.

Strompfová, V., M. Marciňáková, M. Simonová, et al. 2006. Application of potential probiotic *Lactobacillus fermentum* AD1 strain in healthy dogs. *Anaerobe* 12: 75–79.

Swanson, K.S., C.M. Grieshop, E.A. Flickinger, et al. 2002. Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. *Journal of Nutrition* 132: 3721–3731.

Tzortzis, G., M.L.A. Baillon, G.R. Gibson, et al. 2004a. Modulation of anti-pathogenic activity in canine-derived *Lactobacillus* species by carbohydrate growth substrate. *Journal of Applied Microbiology* 96: 552–559.

Tzortzis, G., A.K. Goulas, M.L.A. Baillon, et al. 2004b. In vitro evaluation of the fermentation properties of galactooligosaccharides synthesized by α-galactosidase from *Lactobacillus reuteri*. *Applied Microbiology and Biotechnology* 64: 106–111.

Vester, B.M., and G.C. Fahey Jr. 2009. Prebiotics and probiotics in companion animal nutrition. In *Handbook of prebiotics and probiotics: Health benefits and food applications*, ed. S.S. Cho and T. Finocchiaro. Boca Raton: CRC Press.

Vier, J.K., R. Knorr, C. Cavadini, et al. 2007. Effect of supplementation with *Enterococcus faecium* (SF68) on immune functions in cats. *Veterinary Therapeutics* 8: 229–238.

Weese, J.S. 2002. Microbiological evaluation of commercial probiotics. *Journal of the American Veterinary Medical Association* 220: 794–797.

Weese, J.S. 2003. Evaluation of deficiencies in labeling of commercial probiotics. *The Canadian Veterinary Journal* 44: 982–983.

Weese, J.S., and M.E.C. Anderson. 2002. Preliminary evaluation of *Lactobacillus rhamnosus* strain GG, a potential probiotic in dogs. *The Canadian Veterinary Journal* 43: 771–774.

Weese, J.S., and L. Arroyo. 2003. Bacterial evaluation of dog and cat diets that claim to contain probiotics. *The Canadian Veterinary Journal* 44: 212–215.

Chapter 7
Current Perspectives on Probiotics in Poultry Preharvest Food Safety

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Abstract In the United States, consumption of chicken and turkey continues to increase and there has been a shift in the dynamics of poultry production. With these significant changes, effective strategies for intervention are required to maintain the food safety of these products to protect public health. In recent years, there have been growing concerns regarding antibiotic resistance, prohibition of growth promoters, and consumer demand for antibiotic or chemical-free produce. Such factors are critical in identifying potentially safe and alternative strategies in bird production. In this context, considering the use of probiotics in poultry production would be prudent as food safety remains a contemporary issue. Their implementation has great potential in delivering promising results by reducing the intestinal pathogenic load and thereby reducing the subsequent contamination in poultry production. Several mechanisms of action have been proposed including resistance to colonization, competitive exclusion, production of toxic and inhibitory compounds, competition for nutrients and stimulation of the immune system. Probiotics also offer potential host-protective health effects and bird growth benefits by modulating the gut microflora.

7.1 Introduction

Food-borne illness and its implications for the food industry and the general public is a concern, and this impact could be reduced by establishing effective, novel interventions to enhance food safety in a "farm to fork" approach (Oliver et al. 2009).

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Conventionally, most of the efforts for controlling the pathogens have focused on the period after harvesting (postharvest); however, limiting the spread of food-borne pathogens prior to harvest (as they are reservoirs/asymptomatic carriers of several food-borne pathogens) has increasingly gained interest and focus among food safety researchers, policy makers, government officials [Food Safety and Inspection Service/U.S. Department of Agriculture (FSIS/USDA)], and consumers (Tuohy et al. 2005; Choct 2009; FSIS/USDA 2010). Preharvest food safety is now being considered equally essential to protect the food supply as food-borne pathogens not only can originate from birds entering slaughter but cross contamination may occur with workers or machinery in the processing environment or by direct contact with feces or digesta from the intestinal tract (Corry et al. 2002; Rasschaert et al. 2006; Rasschaert et al. 2008).

7.2 Food-Borne Pathogens Associated with Poultry

The Centers for Disease Control and Prevention (CDC) estimated that there are 76 million cases of food-borne diseases with 325,000 hospitalizations and 5,000 deaths occurring every year in the United States (CDC 2009). Most of these food-borne illness/outbreaks have been linked to contaminated poultry products or contact with food animals, waste, and enteric pathogens in poultry (Doyle and Erickson 2006). Food-borne pathogens mainly transmitted through raw and processed poultry products are *Salmonella* and *Campylobacter* (Mead et al. 1999; Bryan 2001; Park et al. 2008)*.* In 2008, the incidences of food-borne diseases associated with *Salmonella* and *Campylobacter* were 16.2% and 12.68%, respectively (Vugia et al. 2009). In addition to the common bacterial pathogens, zoonotic parasitic infestations such as *Trichinella spiralis* and *Toxoplasma gondii* can pose health risks to consumers (Gebreyes et al. 2008).

Salmonellosis is the second most commonly reported food-borne disease associated with consumption of meat, poultry, eggs, milk, and seafood in the United States (Vugia et al. 2007). According to CDC estimates, every year in the United States there are approximately 1.4 million cases of salmonellosis that are responsible for 17,000 hospitalizations and 585 deaths (Mead et al. 1999; Voetsch et al. 2004). Farm animals such as chicken and turkey represent a major reservoir of *Salmonella* and can also act as asymptomatic carriers in the absence of clinical disease (Oliver et al. 2009).

Salmonella in chickens can contaminate meat and eggs and have become a persistent problem associated with the poultry industry in the United States on an annual basis (USDA 2007a, b). Factors such as age, *Salmonella* serotype and the initial challenge dose level, stress, presence of feed additives (antimicrobials and

anticoccidials), survival through low pH of the crop, competition with gut microflora, and the presence of compatible colonization sites influences the susceptibility of poultry to *Salmonella* colonization (Bailey 1988).

Campylobacter jejuni is one of the major food-borne agents associated with diarrhea and gastroenteritis and represents a major concern to the poultry industry (Altekruse et al. 1999; Newell and Fearnly 2003). The prevalence of *Campylobacter* in poultry in the United States is 32–53% (Miller and Mandrell 2005). Over the years, *Campylobacter* has evolved and adapted to colonize in poultry intestine, which can pose serious public health hazards (Heuer et al. 2001; Newell and Davison 2003).

According to the preliminary surveillance data by FoodNet in 2008, the estimated incidences associated with *Salmonella*, *Campylobacter*, and other food-borne pathogens did not change significantly when compared to the previous 3 years (Vugia et al. 2009). This reinstates the importance and demand for effective control strategies and interventions to produce wholesome food products.

7.3 Preharvest Control Strategies

Developing interventions that have potential in reducing pathogens substantially in the live animal can improve food security and safety (Loneragan and Brashears 2005; Ricke and Jones 2010). A wide range of intervention strategies have been developed to reduce the burden of food-borne pathogens in poultry, including genetic selection of animals that are resistant to colonization, sanitation practices, additives (feed or water), and biological treatments that directly or indirectly inactivate the pathogen within the host (Doyle and Erickson 2006). However, use of antibiotics and chemotherapeutics in prophylactic doses for prolonged periods has led to concerns across the world regarding cross resistance and multiple antibiotic resistances among food-borne pathogens (Mathur and Singh 2005). Furthermore, use of antibiotics as growth promoters in feed to reduce pathogens affects the export of meat and poultry products to European countries (EC 2001; EC 2003). This, then, has generated interest in the development of novel, innovative, and safe alternatives that would boost natural defense mechanisms, including such interventions as acidification of feed by organic acids and feeding probiotic organisms and prebiotic compounds (Williams et al. 2001; Patterson and Burkholder 2003; Oliver et al. 2009; Ricke and Jones 2010). In addition to food safety issues, high protein prices and environmental concerns have caused the poultry industry to consider adopting feed supplements such as probiotics that would positively influence the birds' performance by modulating the gut microflora (Tuohy et al. 2005).

7.4 Chicken Gut Microflora

The chicken gut [also referred as the digestive tract or gastrointestinal (GI) tract] begins with the mouth and ends at the cloaca with several important organs in between [e.g., esophagus, crop, proventriculus (true stomach), gizzard/ventriculus, small intestine, ceca, large intestine]. Based on the microflora dynamics and their colonization perspective, the poultry intestine can be divided into three parts: (1) duodenum and the small intestine, where bacteria numbers are relatively low ($<10^8$/g); (2) ceca, the major site of bacterial colonization and microbial fermentation (10^{11}–10^{12}/g; wet weight); (3) large intestine (Barnes 1972; Barnes et al. 1972).

The GI tract and its associated tissues in poultry during hatching time are relatively sterile and underdeveloped (Cressman 2009). However, as the chick or poult grows, the GI tract provides the required conditions for bacterial colonization, including attachment sites, optimal pH, substrate/nutrients, and waste removal. At this stage, healthy broilers exhibit significant changes, such as more rapid proportional weight increases of GI tissues when compared to total body mass and increased villi volume (three- to fivefold) between the 2nd and 14th day and crypt depth (two- to threefold by day 14) (Uni et al. 1998). Similar increases have also been observed in poults although not to the same extent (Uni et al. 1999).

Development of the normal GI microflora of poultry has been studied extensively in specific pathogen-free (SPF) chickens, which should be unbiased owing to the absence of competitive microflora. The use of SPF birds is more advantageous than using conventionally raised chickens as there is no risk of additional infectious agents such as viruses and parasites that may be present in the latter (Coloe et al. 1984). In the same study on the development of normal gut microflora in SPF chickens, no bacteria were detected at hatching (day 1); in addition, significant levels (10^8 cfu/g) of facultative anaerobes such as fecal streptococci and coliforms had developed by day 3 and *Proteus* sp. ($>10^7$ cfu/g) by day 7 in the cecum.

In poultry, major sites of colonization by gut microflora in GI tract are the crop, proventriculus, gizzard, small intestine, colon, and ceca (Chichlowski et al. 2007a; Gaskins et al. 2002; Heczko et al. 2000; Rastall 2004). Normal GI microflora and representative bacteria in various parts of the healthy chicken GI tract are presented in Table 7.1. In the proximal part of the intestine (crop, gizzard, proventriculus) there are usually low numbers of anaerobic bacteria due to the presence of oxygen, low luminal pH, and hydrochloric acid originating from the proventriculus (Rastall 2004). Despite these unfavorable conditions, lactobacilli can survive in the chicken crop owing to surface receptors on lactobacilli that have the ability to adhere to the squamous epithelial cells of the crop to be retained in high numbers (10^7–0^8) (Fuller 2001) and exhibit stable, persistent, host-specific adhesion effects (Fuller 1973). Consequently, a predominance of lactobacilli in the crop results in the production of lactic acid, which can reduce the number of *Escherichia coli* and *Salmonella* significantly during contamination (Fuller 1977; Durant et al. 1999, 2000).

Microbial colonization of the poultry GI tract starts with microbial contact from the eggshell, feed, and other environmental sources immediately after hatching

Table 7.1 Gut microflora of the chicken with predominant microflora observed in primary gastro-intestinal sections

GI tract section	pH[a]	Density[b] (cfu/g)	Representative Gut microflora[c]	Indigenous microflora with potential probiotic properties
Crop, gizzard, duodenum	3.0–6.0	10^3–10^5	Lactobacilli, coliforms, Streptococci	Acid-tolerant lactobacilli
Small intestine	6.5–7.5	10^8–10^9	Facultative anaerobes (lactobacilli, streptococci, enterobacteria), anaerobes (*Bifidobacterium* spp., *Bacteroides* spp., *Clostridia* spp.), *Eubacterium*, coliforms	Lactobacilli
Ceca	7.0–7.5	10^{10}–10^{12}	Facultative anaerobes (streptococci, coliforms, *Proteus*), obligate anaerobes (*Clostridium*), *Eubacterium*, *Bacteroides*, *Lactobacillus*, *Methanobrevibacter woesei*	*Bifidobacterium*

[a] Adapted from Chandrasekhar (2009)
[b] Barnes et al. (1972), Coloe et al. (1984), Fuller (2001), Chichlowski et al. (2007b), and Saengkerdsub et al. (2007a)

(Cressman 2009). Normal microflora colonize the GI tract beginning at the early posthatch period, develop a biological association with the host, and can have a significant impact on the uptake and utilization of energy and nutrients (Choct et al. 1996; Smits et al. 1997; Apajalahti and Bedford 2000; Torok et al. 2007). Development of the small intestinal microflora is observed during the first 2 weeks of the posthatching period until several weeks after hatching (Ochi et al. 1964; Smith 1965; Smirnov et al. 2006). Immediately after hatching, there is evidence that bacteria, particularly streptococci and enterobacteria, multiply initially in the ceca and spread throughout the alimentary tract within 24 h (Smith 1965). Lactobacilli can become established by the third day, whereas the streptococci and enterobacteria slowly decline in the GI tract except in the ceca (Barnes et al. 1972). By 2 weeks of age, lactobacilli became the predominant microflora with occasional streptococci and enterobacteria in the duodenum and lower portions of the small intestine (Barnes et al. 1972). In the cecum, *Bifidobacterium* becomes established as the predominant microflora by 30 days (Ochi et al. 1964). Recent evidence based on real-time polymerase chain reaction (PCR) analyses of feces from 3- to 12-day-old broilers also indicates the presence of methanogens in these young birds (Saengkerdsub et al. 2007b). In adult birds most of these methanogens have been identified as *Methanobrevibacter woesei* (Saengkerdsub et al. 2007a). Overall, the composition of the microflora undergoes major changes during the time of hatching, and the anaerobic microflora becomes established, which requires significant amounts of substrates such as carbohydrates (Apajalahti et al. 2002).

The diverse microbial community profile thus developed over time can be identified through molecular techniques such as denatured gradient gel electrophoresis (DGGE), percent guanine-cytosine (% G+C) profiling, and 16S rDNA sequencing (Apajalahti et al. 2002; Gong et al. 2002; Hanning and Ricke 2011; Holben et al. 2002; Zhu et al. 2002). In studies based on combination of% G+C profiling and 16S rDNA sequencing and using certain criteria described by Maidak et al. (1999), it was concluded that: (1) only 10% of the GI bacteria are previously known bacterial species; (2) thirty five percent represent previously unknown species within a known bacterial genus; (3) the remaining 55% represent bacteria for which even the genus is completely unknown. A total of 640 species and 140 bacterial genera have been tentatively identified in the chicken GI tract (Apajalahti et al. 2004).

The microbial community profile in the chicken GI tract is chiefly influenced by the diet (grain base) and the age of the bird (Barnes et al. 1972). Apajalahati and Bedford (2000) studied the effect of grains (wheat, corn, rye) on the microbial community profile and concluded that incorporation of rye in the diet increased the abundance of bacteria with a 35–40% G+C content when compared to wheat and corn. Although this study did not identify individual bacteria, the authors conclude that incorporation of diets with corn favored low% G+C microorganisms (e.g., *Clostridia*, *Campylobacter*), whereas the wheat-based diets favored higher% G + C microorganisms (e.g., *Propionibacteria*, *Bifidobacteria*). In addition to diets, processing of grains has significant effects: Differently processed diets favored different bacteria in the GI tract of the chicken regardless of whether the dietary mix originated from the same raw material (Apajalahti et al. 2001). Furthermore, anaerobes and lactobacilli were found to be significantly lower in gizzards of broilers fed sorghum- and wheat-based diets when compared to broilers raised on barley and maize diets (Shakouri et al. 2008). Similar differences were observed in the cecum, whereas in the ileum there was no effect of grains on anaerobic and lactobacilli populations (Shakouri et al. 2008). Supplementation of the diets with fats and their source has been observed to influence the microbiota structure (Knarreborg et al. 2002; Dänicke et al. 1999). Knarreborg et al. (2002) studied the effect of animal- and plant-derived fats on the microbiota in the ileum of broilers (14–21 days) and reported that the source of dietary fat significantly altered the viable populations of *Clostridium perfringens* whereas *Lactobacillus* species were not affected. Dänicke et al. (1999) demonstrated that broilers fed diets with beef tallow compared to soybean oil had significantly more Gram-positive cocci in the crop, jejunum, and ileum (1.18, 1.05, 1.36, and 2.10 cfu/$\log_{10}$ higher) at day 16. *Enterobacter* was substantially higher in the crop and duodenum (1.05 and 1.30 cfu/$\log_{10}$ higher, respectively) in birds fed with soybean oil; and the total number of anaerobes did not vary substantially across intestinal segments based on the source of fat.

7.4.1   Microflora Changes in Adult Chicken

The age of the bird also influences colonization and susceptibility to infectious agents in the GI tract (Corrier et al. 1999; Apajalahti et al. 2004). Even though gut

microflora in the normal adult chicken constitutes sufficient microbial complexity and is relatively resistant to enteric pathogens, stress associated conditions can make the host susceptible to pathogen infection and colonization (Ricke 2003b; Ricke et al. 2004; Dunkley et al. 2007a, b, d; Dunkley et al. 2009; Norberg et al. 2010). Feed withdrawal was historically followed as the standard practice to induce molting and is a current practice in broilers prior to shipping to clear fecal content in the GI tract and reduce the potential fecal contamination of carcasses (Ricke 2003b; Appleby et al. 2004; Park et al. 2008; Doyle and Erickson 2006). Although this practice has reduced the number of carcasses with fecal contamination, it can significantly increase the *Salmonella* and *Campylobacter* populations in broiler crops (May and Lott 1990; Ramirez et al. 1997; Byrd et al. 1998). Artificial molting in laying hens by withdrawing feed can increase intestinal shedding and dissemination of *Salmonella* Enteritidis to internal organs (ceca, spleen, liver, ovary), thus potentially increasing the public health incidence and susceptibility of salmonellosis through contaminated eggs (Holt et al. 1995; Corrier et al. 1997; Durant et al. 1999; Ricke 2003b; Dunkley et al. 2007c, 2009). This can be due to decreases and shifts in beneficial microflora and its host-protective activities such as microbial fermentation to produce volatile fatty acids (VFAs) in the host's GI tract (Ricke 2003a; Ricke et al. 2004; Dunkley et al. 2007d; Dunkley et al. 2009).

In adult birds there is increasing evidence that providing substrates for fermentation is sufficient to retain protective GI tract microflora that minimizes colonization by food-borne pathogens such as *Salmonella* (Ricke 2003b; Dunkley et al. 2009; Norberg et al. 2010). In layers, feeding alternative low-energy molt induction diets that are rich in fermentable dietary fiber such as alfalfa have been used successfully to produce short-chain fatty acids (SCFAs) in the cecum, thereby reducing the colonization of *S.* Enteritidis and retaining beneficial microflora (Ricke 2003a; Woodward et al. 2005; Dunkley et al. 2007d, 2009; Norberg et al. 2010). Feeding alfalfa crumble diets to laying hens reduced the colonization of *S.* Enteritidis by various mechanisms: It reduced the virulence expression of *Salmonella* virulence gene regulator *hilA* response compared to the feed withdrawal *hilA* levels associated with stress (Dunkley et al. 2007c), and it increased production of SCFAs, which also may limit *Salmonella* (Dunkley et al. 2007d). Furthermore, feeding alfalfa diets favorably influenced some of the physiological metabolites and stress indicators, such as total protein, uric acid, calcium, triglyceride concentration levels, heterophil to lymphocyte ratios, and α_1-acid glycoprotein (AGP) levels that accompany feed withdrawal stress conditions (Dunkley et al. 2007a, b).

In addition, alternative diet regimens containing wheat middlings have been successfully used as molt inducers to limit *Salmonella* colonization (Seo et al. 2001). Similarly, glucose-based treatments and their commercial products, such as D-glucose polymer (maltodextrin), with added salts and vitamins can reduce the microbial load of *S.* Typhimurium (in the crop) or *Campylobacter* infection (Hinton et al. 2000; Northcutt et al. 2003). Addition of zinc acetate (10,000 ppm) in laying hen diets has been shown to reduce *S.* Enteritidis colonization in crop during induced molt, but it depends on the zinc concentration (Moore et al. 2004; Park et al. 2008). Furthermore, incorporation of carbon substrates such as lactose into drinking water in conjunction with a feed withdrawal molt has been shown to improve the resistance to *S.* Enteritidis

colonization by enhancing the fermentative cecal population without dietary intervention (Corrier et al. 1997). In broilers, feeding alternative diets consisting of semisynthetic ingredients during the last 72 h before slaughter yielded less feed intake and lower live weights (0.24% less per hour) than broilers subjected to feed withdrawal, thereby decreasing the gut contents that would ultimately be involved in contamination of the carcasses during evisceration (Nijdam et al. 2006).

7.5 Probiotics

Probiotic in Greek means "for life" (Gibson and Fuller 2000) and here is defined as "a dietary supplement of living bacteria that exhibits beneficial effects in the host through its effect in the intestinal tract" (Roberfroid 2000). Probiotic supplementation is a strategy that promotes the growth of beneficial microorganisms that are competitive or antagonistic to food-borne pathogens. Conceptionally, this infers the establishment of microbial niches in the gut that prevent the colonization of pathogenic bacteria.

7.5.1 Historical Background

For centuries, microorganisms have been used in food processing such as for fermented dairy and meat products (Johnson and Steele 2007; Ricke et al. 2007). Metchinkoff (1907) proposed that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes." Consumption of dairy foods such as fermented milk, buttermilk, and yogurt is associated with health benefits and longevity in Bulgarian peasant populations; and Metchinkoff (1908) proposed the scientific reasons for their beneficial effects. The term probiotic was defined by Parker (1974) as "organisms and substances that contribute to intestinal microbial balance." Fuller (1989) attempted to refine the definition of probiotic as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." In later years, there were several attempts to further modify the definition of probiotics by including products in addition to microorganisms or their preparations, proposing the phrase "alteration of microflora" over enhancing the beneficial effects of microflora and by redefining the term indigenous microflora (Havenaar and Huis in't veld 1992; Salminen et al. 1996; Schaafsma 1996).

7.5.2 Regulatory Considerations of Probiotics

In the United States, probiotics used as feed supplements are required to have "generally recognized as safe" (GRAS) status, which is regulated by the U.S. Food and Drug Administration. Feed supplements claiming the presence of probiotic bacteria

Table 7.2 Overview of labeling guidelines/information and selection criteria that should be followed by commercial probiotic feed or supplements (International Probiotic association & World Health Organization) (Dash 2009; Przyrembel 2001; Reid 2005)

Step	Approach	Points of consideration	References
1	Identification and confirmation of probiotics	Genus, species, and strain identification through genotype and phenotype methods	Wang et al. (2002), Gardiner et al. (2002), and Burton et al. (2003)
2	Determination and validation of probiotic stability	In vitro tests should be performed to confirm the ability to adhere to surfaces, inhibit growth, and attachment of pathogens and resistance to the environmental stress, etc.	Reid et al. (1987) and Conway et al. (1987)
3	Safety of probiotics	Validation of newly introduced probiotics and their safety through in vitro tests, laboratory animal feeding trials, and genomic sequence	Marteau (2002)
4	Efficacy of probiotic on host health	Evaluation of the health benefits incurred by probiotic usage and compare with a placebo trial	Reid (2005)
5	Health claims and labeling	Involves labeling guidelines and specific health claims: genus, specie,s and functionality of the strain on the label	Dash (2009)
		Strength of the probiotic strain (cfu/ml or g) in the product	
		Serving size and effective dose of probiotic	
		Total servings per container	
		Proven health claims (scientific research validation)	
		Storage conditions	
		Manufactureer's name and contact address	
		Manufacturer's lot number and expiration date	

must cite the name of the exact taxonomical species of probiotic(s) to avoid misidentification. Manufacturers should also provide the "best before" date of the product with recommended storage conditions; and the strength of the probiotic should match what is declared on the label with a maximum deviation of one or two logarithmic units (Czinn and Blanchard 2009).

Although there are numerous advantages of probiotic feed supplements, their usage is often associated with adverse side effects, unreliability, and unconfirmed clinical significance (Przyrembel 2001). To ensure product equality, safety, reliability, and appropriate usage, the United Nations Food and Agricultural Organization (FAO) formulated guidelines that led to the development of operating standards in 2002 (Reid 2005). An overview of guidelines for evaluating the commercial probiotics including labeling requirements is presented in Table 7.2. Furthermore, these

Table 7.3 Ideal characteristics of optimal probiotics

Characteristic	Reference
Nonpathogenic and nontoxic; be host origin	Lan et al. (2003)
Resistant and persistent to stress, processing and storage, gastric acid and bile	Rastall (2004)
Suitable adherence factors to attach in intestinal epithelium or mucus and compete for binding sites	Chichlowski et al. (2007a)
Produce toxic conditions and antimicrobial/inhibitory compounds (e.g., VFAs, low pH, bacteriocins)	Saavedra (1995)
Stimulate/modulate host immune system	Saavedra et al. (1994)
Alter microbial activities by demonstrating microbial antagonism	Gibson et al. (1997)
Genetically stable and viable at high populations	

guidelines and recommendations are considered essential to identify and accurately define the health benefits claimed by the probiotics (FAO/WHO 2002). In view of numerous probiotics in the current market claiming various health benefits, they should be selected and used based on the appropriate criteria as discussed in the following section.

7.5.3   Selection Criteria of Probiotic Strains

Identifying appropriate probiotic strains to achieve maximum beneficial effects in the host is a challenging task. Ideal characteristics for an optimal probiotic are presented in Table 7.3. Selection of suitable probiotic strains is influenced by factors such as the colonizing ability of the microflora in the gut, resistance to antibacterial factors such as hydrochloric acid in the proventriculus and gizzard, bile acids in the small intestine and SCFAs in the ceca, stability and safety, and the ability to produce antibacterial compounds. Proper criteria as discussed in the previous section (Table 7.2) should be followed for safety, production, administration, application, survival, and colonization in the host (Dash 2009).

7.6   Potential Mechanisms of Action of Probiotics in Poultry

Ever since the concept of probiotics was introduced, there has been a drastic change in the perspectives regarding the composition and knowledge of the gut microflora (both obligate and facultative anaerobes) and their mechanisms of action in the concerned host. Microorganisms in the host are present as diverse and complex communities that are dependent on each other and their environment, in contrast to the general opinion that they are independent from surrounding bacteria (Nisbet 2002; Apajalahti et al. 2004; Ricke et al. 2004). Most of the bacteria have certain growth requirements, as yet not completely identified, that are satisfied by their natural habitats and other synergistic bacterial species living in the same community (Nisbet et al. 1996a, b; Apajalahti et al. 2004).

Introduction of probiotic microflora into the host gut should lead to the formation of a complex ecosystem and generation of additional microbial interactions among the gut microflora (Guillot 2009). However, these interactions should be balanced, well-established biological defenses against pathogenic organisms. There are several mechanisms of action through which probiotics can act in the host, some of which are discussed in the following sections.

7.6.1 Immune System Stimulation

Most studies have demonstrated the effect of probiotics on systemic immunity of the host. Incorporation of probiotics in the animal diet can stimulate the immune system by migrating through the intestinal wall as viable cells and multiply to a limited extent, causing production of immunogenic compounds, and mediating down-regulation of specific signaling pathways (Fuller 1975; Havenaar and Spanhaak 1994; Schiffrin et al. 1995; Yurong et al. 2005). Consequently, stimulated immunity may manifest as enhanced macrophage activity and a systemic antibody response through enhanced production of immunoglobulins (IgG, IgM), interferons, IgA levels at mucosal surfaces, and expression of various pro- and antiinflammatory cytokines. Administration of probiotics can also lead to increased IgA levels in the lumen; IgA-, IgM-, and IgG-producing cells; and T cells in cecal tonsils (Yurong et al. 2005). Similarly, probiotic administration has been shown to increase natural antibodies against several antigens in both gut and serum (Haghighi et al. 2006). Furthermore, administration of probiotics in chickens has been demonstrated to elicit significant increases in the oxidative burst and degranulation of heterophils (Farnell et al. 2006).

7.6.2 Competitive Exclusion

This initial concept of competitive exclusion (CE) was based on the Metchinkoff principle (1907). CE refers to the physical blocking of intestinal pathogens by probiotic bacteria owing to their ability to colonize niches within the intestinal tract such as intestinal villi and colonic crypts (Duggan et al. 2002). Nurmi and Rantala (1973) and Rantala and Nurmi (1973) successfully demonstrated that chicks (1–2 days old) developed resistance to *Salmonella infantis* colonization when they were inoculated with a suspension of "gut contents" from healthy adult roosters. This mechanism was referred to as CE by Lloyd et al. (1974) and was first applied to poultry. This study initiated worldwide research for potential CE cultures (Pivnick and Nurmi 1982). Competitive exclusion application involves the inclusion of nonpathogenic bacterial culture (single or several strains through oral administration) to the intestinal tract of food animals to reduce the colonization or populations of pathogenic bacteria in the GI tract (Nurmi et al. 1992; Steer et al. 2000).

The exact mechanisms by which the probiotic bacteria prevent the colonization of pathogens are typically considered organism-specific. *Lactobacillus plantarum* induces transcription and excretion of the mucins MUC2 and MUC3 from goblet cells and thus inhibits the adherence of enteropathogenic *Escherichia coli* (EPEC) to the intestinal surface (Fooks and Gibson 2002). Other mechanisms include changes in the physical microenvironment of the intestinal tract by preventing or competing with pathogenic bacteria for nutrients, growth, and function (Cummings and Macfarlane 1997) as well as production of small antimicrobial molecules such as VFAs, lactic acid, or bacteriocins (Kohler et al. 2002).

Selection of CE microflora (especially cecal microflora) depends on their ability to ferment specific carbohydrates in the form of dietary lactose and mannose or other compounds to produce protective compounds such as VFAs, which in turn reduce the pathogenic microorganisms in the gut (Oyofo et al. 1989a, b, c; Hinton et al. 1992; Nisbet et al. 1993; Ricke 2003a). In addition, availability of growth-limiting amino acids such as serine, oxidation-reduction potential, and level of anaerobiosis in the cecum can play an important role in reducing intestinal nonindigenous organisms in the host (Goren et al. 1984; Nisbet et al. 1993; Ha et al. 1994, 1995; Nisbet et al. 1994; Ricke et al. 2004).

7.6.3 Alter the Intestinal pH

The presence of probiotic microorganisms in the intestine produces organic acid end-products such as VFAs and lactic acid (Gibson 1999). These weak organic acids reduce the pH, thereby potentially creating unfavorable conditions for survival of pathogenic bacteria such as *Escherichia coli* and *Salmonella* (Ricke 2003a; Marteau et al. 2004; Van Immerseel et al. 2010). VFAs absorbed from the colon serve two purposes: stimulating water and electrolyte absorption and providing energy (60–70%) from the bacterial fermentation (Marteau et al. 2004). VFAs are also involved in hepatic regulation of lipid and carbohydrates, which can serve as energy substrates to vital organs of the host such as the heart, kidney, brain, and muscle (Meghrous et al. 1990).

7.6.4 Colonizing Ability

Probiotic bacteria can colonize three areas in the GI tract: surface, cecal epithelial surface, and the colonic epithelial surfaces (Yamauchi and Snel 2000). In general, there are at least three microenvironment niches to each of the aforementioned areas: the digesta, surface enterocytes, cecum and colon, the mucus blanket, and the epithelial surface (Chichlowski et al. 2007a).

Probiotic bacteria such as *Lactobacillus* and *Enterococcus* have the ability to colonize the gut of axenic (no microflora) and gnotoxenic (consists of specific or

known microflora) chickens (Guillot 1998). Spores of *Bacillus* cannot colonize the gut in axenic and gnotobiotic animals and are referred to as transients. The colonizing ability is measured by colony forming units (cfu); in poultry this process begins at the beak and progresses distally to the colon (Simon et al. 2004). Factors influencing successful probiotic colonization include the type of probiotic strain and its host specifity, stability of the probiotic strain, dose and frequency of administration, the health and nutritional status, age, stress level, and genetics of the host (Mason et al. 2005).

The first step in colonization of the probiotic bacteria is their attachment to the plasmalemma of the enterocyte so they can resist subsequent actions for removal from the gut through peristalsis and mixing of the digesta and mucus layer (Chichlowski et al. 2007a). Lactobacilli that originate from ingested food or are shed from epithelial surfaces in poultry can permeate the entire digestive tract (Henriksson et al. 1991; Servin and Coconnier 2003).

The ultimate beneficial effect of probiotic bacterial colonization is to prevent the adherence of pathogenic bacteria. Briandet et al. (1999) reported that bifidobacteria and lactobacilli produced dose-dependent inhibition of adherence of enterotoxigenic *Escherichia coli* (ETEC), EPEC, and *S.* Typhimurium. In vitro techniques such as microbial adhesion to solvents (MATS) can be used to estimate the affinity of the bacterial cells to polar and nonpolar solvents (Wadstrom et al. 1987). Bomba et al. (2002) demonstrated that hydrophobic interactions are more predominant than hydrophilic interactions in bacterial attachment to intestinal epithelial cells. However, lactobacilli have strong affinity toward a polar solvent (maximum at pH 7), which suggests that it has inclination toward hydrophilic associations with cellular surfaces (Huang and Adams 2003). Dietary inclusion of polyunsaturated fatty acids (PUFAs) can affect the attachment sites for the GI microbiota by modulating the chemical composition of fatty acids in the intestinal wall, thus altering its hydrophobicity (Fooks and Gibson 2002). Probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* reduce the oxidation–reduction potential in the gut and provide a favorable environment suitable for colonization (Cummings and Macfarlane 1997).

7.6.5 *Maintenance of Epithelial Barrier Integrity*

Probiotic bacteria are reported to enhance the maintenance and function of the epithelial barrier (Madsen et al. 2001) which are believed to occur through two major mechanisms. With the first mechanism, probiotics increase the basal luminal mucin content through up-regulation of mucin and *MUC2* gene expression (Caballero-Franco et al. 2007) and may increase growth and maturation of goblet cells through metabolites produced from intestinal bacterial fermentation (Chichlowski et al. 2007b). In vitro studies involving *Lactobacillus* have also demonstrated increased production of mucin (Montalto et al. 2004). This secreted mucin serves as a "mucous blanket" and is composed of numerous small associated proteins, glycoproteins,

lipids, and glycolipids (Caballero-Franco et al. 2007). It also contains soluble receptors that recognize specific adhesion proteins, which in turn facilitate bacterial attachment (Chichlowski et al. 2007b).

The second mechanism wherein probiotic strains may be involved is alteration of the permeability of tight junctions (*Zonula occludens*) to strengthen the biological barrier in the intestinal wall (Shen et al. 2006). These tight junctions form an unbroken, continuous barrier that prevents infectious bacterial entrance and penetration by large molecules of digesta (Chichlowski et al. 2007b). The permeability function of tight junctions is modulated by zonulin, a molecule involved in fluids, macromolecules, and leukocyte movement from the bloodstream into and out of the intestinal lumen (Shen et al. 2006). Buts et al. (2002) reported a protective effect of *Lactobacillus* on zonulin following treatment with nonsteroidal antiinflammatory drug administration in vitro. Shen et al. (2006) demonstrated that more intact epithelial cell tight junctions occur after probiotic treatment, but the exact mechanisms responsible for this observation were not clear in this study.

7.7 Applications of Probiotics in Poultry Preharvest Food Safety

7.7.1 Introduction

Numerous studies have been conducted and have highlighted the beneficial effects of using probiotic feed supplements to enhance the performance and stimulate immune responses in poultry (Table 7.4). In general, food animals are often exposed to stress due to physiological (age, health status), psychological (dietary changes), and environmental (climate, management) factors. This may lead to dysfunction and an increase in the permeability of intestinal protective barriers that often results in changes in the intestinal microbial composition (e.g., decrease in bifidobacteria and lactobacilli) and an increase in susceptibility to enteric pathogens (Si et al. 2004). Although probiotic organisms such as *Pediococcus* and *Saccharomyces* are less commonly used in animal feeds, they can modulate the establishment of lymphocyte populations and IgA secretions in the gut and reduce translocation to mesenteric lymph nodes followed by *E. coli* ETEC infection (Lessard et al. 2009).

Poultry management practices such as high stocking densities, transportation, and nutritional imbalances or regimen may predispose stress that would ultimately affect the host's immune system and colonization of pathogenic bacteria in the gut and lead to potential food safety issues (Virden and Kidd 2009). Supplementation of probiotics in poultry diets has been considered an effective tool to maintain a healthy intestinal microbiota, thereby improving the growth performance and reducing intestinal pathogens (Jin et al. 1996). Factors affecting the functionality or efficacy of probiotic supplementation are the route of administration (vent, feed, water) and stage of the life cycle (Timmerman et al. 2006). Probiotic supplementations can be administered

through powders, liquid suspensions, or sprays in feed or water as well as *in ovo* methods where the shell membrane of the air cell is inoculated with the probiotic culture after 18 days of incubation for early gut colonization (Fuller 2001).

7.7.2 Role of Probiotics: Beneficial Effects

Probiotic bacteria (e.g., lactobacilli and bifidobacteria) have been identified in poultry that can modulate the immune system of the host by stimulating certain subsets of the immune system to produce cytokines (Christensen et al. 2002; Lammers et al. 2003; Maassen et al. 2000). Dalloul et al. (2003a, b) demonstrated that administration of probiotics results in secretion of cytokines and changes in the lymphoid cells in the chicken gut that may ultimately provide immunity against *Eimeria acervulina*. However, a precise understanding of the effect of probiotics on the induction of systemic antibody response is not well established (Haghighi et al. 2006). Beneficial effects of probiotic supplementation in poultry is mainly attributed to CE, which has demonstrated protection against colonization of *Salmonella*, *C. jejuni*, pathogenic *E. coli*, and *C. perfringens* in chicks (Nisbet 2002; Schneitz 2005).

Use of *Lactobacillus* as a probiotic nutritional and health supplement is an increasing trend in the poultry industry as it modulates the immune system of the host and increases overall performance including growth rate, feed conversion ratio, and meat quality (Kalavathy et al. 2003; Mountzouris et al. 2007). Addition of *Lactobacillus* successfully lowered the mortality rate due to necrotic enteritis in 1-day-old chicks (Hofacre et al. 2003). *Lactobacillus*-supplemented poultry diets also significantly reduced *S.* Enteritidis recovery in neonatal chicks (Higgins et al. 2007a, 2008). Other probiotic bacteria, such as *Bacillus cereus* var. *toyo* and *B. subtilis*, suppressed the persistence and colonization of *S.* Enteritidis and *C. perfringens* (La Ragione and Woodward 2003). Broiler chicks fed with a mixture of probiotics (*L. acidophilus*, *L. casei*, *Bifidobacterium thermophilus*, *E. faecium*) lowered *C. jejuni* populations (Willis and Reid 2008).

7.7.3 Commercial Probiotic Supplements

Currently, there are several commercial probiotic supplements consisting of beneficial microorganisms alone or in combination with fermentable carbohydrates (prebiotic compounds) available in the market (Table 7.4). Probiotic microorganisms such as *Lactobacillus* spp., *Enterococcus* spp., *Pediococcus*, and *Bacillus* spp. are commonly found in commercial supplements, with *Lactobacillu*s spp. the predominant group.

Several studies were conducted on *Lactobacilli* spp.-based commercial probiotics [Floramax (FM-B11), Histostat-50, Nutra-Glo] in poultry (Higgins et al. 2005, 2007a, b, 2008). Dietary supplementation of *Lactobacilli* spp. in poults (7 days old)

Table 7.4 List of commercial probiotic in the market with the probiotic cultures and their beneficial effects

Commercial product	Active components	Company	Beneficial effects in the host	References
Poultry Star®	*Enterococcus, Pediococcus, Lactobacillus, Bifidobacterium* (probiotic) and fructooligosaccharides (prebiotic)	Biomin, Herzogenburg, Austria	• Inhibition of pathogens, (*S. enteritidis, S. Typhimurium, S. Choleraesuis, C. jejuni, E. coli* and *Cl. perfringens*) • Immunological activity, VFA's production, stability against acids and bile salts	Sterzo et al. 2007; Mountzouris et al. 2010
BIOMIN Poultry5star	*Lactobacillus reuteri, Enterococcus faecium, Bifidobacterium animalis, Pediococcus acidilactici, Lactobacillus salivarius* (2×10^{12} cfu/kg)	Biomin, Herzogenburg, Austria	• Growth-promoting effect • Modulation of the composition and activities of the cecal microflora • Higher specific microbial glycolytic enzyme activities (α-galactosidase and β-galactosidase) compared with the control	Mountzouris et al. 2007
PremaLac	*Lactobacillus acidophilus, L. casei, Bifidobacterium* and *Enterococcus faecium*	Star-Lab, Clarksdale, MO, USA	• Increased body weight of broilers (0 to 21 days)	Pour and Kermanshahi 2010
Super-BioLicks®	*Leuconostoc* spp. (10^7 CFU/g, *Pichia* spp. (10^7 CFU/g and *Bacillus subtilis* 10^5 CFU/g)	Nippon Formular Feed, Mfg, Yokohama, Japan	• Increase in body weight and growth performance by inducing hypertrophy in intestinal villi and epithelial cells	Khambualai et al. 2010
Bactocell®	Lactic acid bacterial strain, *Pediococcus acidilactici* (10^7 CFU/g	Lallemand Animal Nutrition, Blagnac, France	• Enhanced immune system stimulation and function • Significant increase in antibodies production against Newcastle disease virus (Relative increase in bursa of Fabricius, spleen and thymus)	Alkhalf, Alhaj and Al-Homidan 2010
PREEMPT™	15 facultative anaerobic bacteria and 14 obligate anaerobic bacteria (Competetive exclusion culture, 10^7 CFU/g)	MSBioscience, Madison, WI, USA	• Protection from *Salmonella* colonization by competitive exclusion and increasing the cecal propionic acid concentration	Corrier et al. 1995a, b; Nisbet et al. 1996a, b; Martin et al. 2000

Product	Composition	Manufacturer	Effects	References
Bio-Plus 2B®	2.3×10^8 CFU/g each of *Bacillus licheniformis* and *Bacillus subtilis* spores	Chr. Hansen A/S, Horsholm, Denmark	• Improved live body weight, feed conversion ratio, and antibody response to Newcastle disease virus	Rahimi 2009
Histostat-50®	*Lactobacillus casei* and *L. bulgaricus* (~10^7 CFU/mL)	Alpharma, Fort Lee, NJ, USA	• Increase in body weight, performance	Higgins et al. 2005
Floramax™ (FM-B11)	*L. bulgaricus* (3), *L. fermentum* (3), *L. casei* (2), *L. cellobiosus* (2), and *L. helveticus* (1)*	Pacific Vet Group, Fayetteville, AR, USA; IVS-Wynco LLC, Springdale, AR, USA	• Increased in weight gain in poults • Effective in treating clinical enteritis caused by *Salmonella Seftenburg* when used along with therapeutic antibiotic regimes • Reduced *S.* Enteritidis colonization in cecum of neonatal chicks (1-day old) due to phagocytic action of macrophages • Reduced the incidence of *S.* Typhimurium (60 to 70 %) and *S.* Enteritidis (89 to 95 %) in day-of-hatch broilers	Higgins et al. 2005 Higgins et al. 2007b; Higgins et al. 2008 Higgins et al. 2007a
Toyocerin®	*Bacillus cereus* var. *toyoi* (10^9 viable spores/g product)	Lohmann Animal Health Int., Winslow, ME, USA	• Increased performance (fattening) in broilers and turkeys	Jadamus et al. 2000
Protexin™	7 strains of bacteria (*L. plantarum*, *L. delbrueckii* subsp. *Bulgaricus*, *L. acidophilus*, *L. rhamnosus*, *B. bifidum*, *S. salivarius* subsp. *Thermophilus*, *E. faecium*; 10^{10} cfu/kg each) and two yeasts (*A. oryza*, *C. pintol-opesii*; 10^9 cfu/kg)	Probiotics International Ltd, Lopen Head, UK	• Improved laying performace (egg production and shell weight) in Japenese quails (*Coturnix coturnix Japonica*) • Immunomodualtory effect (Increase in Avian Influenza viral antibodies)	Ayasan et al. 2005; Ghafoor et al. 2005

(continued)

Table 7.4 (continued)

Commercial product	Active components	Company	Beneficial effects in the host	References
Bactocell® + Lactose or Myco® (Mannoseo-ligosaccharides)	Lactic acid bacterial strain, *Pediococcus acidilactici* (10^7 CFU/g) Myco	Lallemand Animal Nutrition, Blagnac, France Probyn International, Lombard, IL, USA	• Bactocell, Lactose, and Myco improved feed conversion ratio (FCR): 3. 2 %, 0.5 %, and 1.6 % respectively • Bactocell® + Lactose improved FCR by 4.2 % • Bactocell® + Myco® improved FCR by 5.34 %	EL-Banna et al. 2010
Bioplus 2B (BP)	Minimum of 3.2×10^9 (CFU/g) *Bacillus subtilis* (CH201) and *Bacillus licheniformis* (CH200)	Chr. Hansen A/S, Horsholm, Denmark	• Increased feed conversion, weight gain. • Significant increase in the goblet cell numbers	Mahdavi et al. 2005; Dizaji and Piromohammadi 2009; Sabatkova et al. 2008
Lacto-Sacc/ Lacto-Sacc Farm pak 2X	Dried *Streptococcus faecium* & *L. acidophilus*, yeast culture (live *S. cerevisiae*, *A. niger*), dried tridhoderma viride fermentation extract (beta glucan)	Alltech, Inc, Springfield, KY, USA	• Improved egg production, egg mass/hen/day, egg weight and feed conversion ratio in broilers	Zeweil et al. 2006
NUTRA-GLO™	*L. acidophilus* fermentation product	Sunrise Supply, LLC, Beach City, OH, USA	• Improved growth rates, feed conversion, egg production	
Aviguard®	Freeze-dried competetive exclusion culture derived from healthy, pathogen free birds	Schering-Plough, Malvern Link, U.K	• Reduced mortality, gross lesions, and performance losses inflicted by the necrotic enteritis infection in broilers • Reduced *Salmonella* colonization without affecting normal antibody response • Significantly reduced colonization by multi-resistant pathogenic *E.coli*	Hofacre et al. 1998 Reynolds 1998 Nakamura et al. 2002

*Number in the parentheses denotes the number of isolates present in the probiotic culture

led to increased weight gain (by at least 18 g on the 21st day); and it effectively treated clinical enteritis caused by *S*. Seftenberg when used along with therapeutic antibiotic regimens (penicillin, Roxarsone, and neomycin) (Higgins et al. 2005). Furthermore, incorporating *Lactobacilli* spp. probiotic cultures into 1-day-old broiler chicks reduced the incidence and colonization of *S*. Enteritidis and *S*. Typhimurium due to phagocytic action of macrophages (Higgins et al. 2007b, 2008). Probiotic supplements containing *Bacillus* spp. (Bio-Plus 2B, Toyocerin) have shown growth-enhancing activity (live weight, feed conversion ratio, fattening) in broilers and turkeys and increased the antibody response to Newcastle disease virus in broilers (Jadamus et al. 2000; Mahdavi et al. 2005; Šabatková et al. 2008; Dizaji and Piromohammadi 2009; Rahimi 2009). Dietary supplementation of the lactic acid strain, *Pediococcus acidilacti* (Bactocell) had reportedly stimulated the immune function of broiler chicks and thus significantly increased antibody levels against Newcastle disease virus (Alkhalf et al. 2010). Administration of commercial probiotic supplements based on competitive exclusion (Aviguard) also significantly reduced colonization of multiresistant pathogenic *E.coli* and *Salmonella* in broilers (Reynolds 1998; Nakamura et al. 2002).

There are several commercial supplements (Poultry Star, PremaLac, PREEMPT, Protexin) containing mixed defined and characterized probiotic cultures in the market. They have been reported to provide diverse benefits to the poultry host such as host protective effects from enteric pathogens (immune stimulation, increased VFA production, reduced colonization, CE) and overall growth performance activities (improved body weight, a better feed conversion ratio) (Ayasan et al. 2005; Sterzo et al. 2007; Mountzouris et al. 2007; Pour and Kermanshahi 2010). Mountzouris et al. (2007) reported that a mixed defined probiotic culture (Biomin Poultry Star) exhibited modulated composition and activities of cecal microflora and displayed higher specific microbial glycolytic enzymatic activity in broilers. In addition to these beneficial effects, some commercial probiotics are well known to protect birds from *Salmonella* colonization by preventing *Salmonella* establishment in the ceca after probiotic (PREEMPT) administration (Corrier et al. 1995a, b; Nisbet et al. 1996a, b; Martin et al. 2000). Furthermore, commercial probiotics consisting of yeasts and beneficial bacteria (Protexin, Lacto-Sacc or Lacto-Sacc Farm pak 2X) have been shown to improve laying performance such as egg production, egg weight, feed conversion ratio (Ayasan et al. 2006; Zeweil et al. 2006), and immune-modulatory activities against avian influenza in broilers (Ghafoor et al. 2005).

Dietary supplementation of probiotic along with prebiotic compounds is also known to elicit several health benefits in poultry. El-Banna et al. (2010) reported an improved feed conversion rate of 4.2% or 5.34% when probiotic (BACTOCELL) was supplemented with lactose or Myco (mannoseoligosaccharide), respectively, in 1-day-old broiler chicks. These combinations (probiotic and prebiotic) also inhibited such enteric pathogens as *S*. Enteritidis, *S*. Typhimurium, *S*. Choleraesuis, *C. jejuni*, and *E. coli* (Sterzo et al. 2007; McReynolds et al. 2009).

7.7.4   Probiotics Inconsistent Responses

Despite numerous reported health benefits in poultry, inconsistent effects have been observed following probiotic administration (Turner et al. 2001). These inconsistent responses are similar or comparable to the effects observed following the administration of conventional antimicrobials. Also, results available from the literature on probiotic treatments often appear to be contradictory. This may be due to variations in the target pathogen, dietary supplementation, and duration of use. Disregarding the environmental and stress status of the animals, the experimental settings are reasons for inconsistent results. Several factors—such as production environment (cleanliness, history of diseases in the farm, health status) (Catala-Gregori et al. 2007), source of the probiotic, number of viable cells in the probiotic and their consistency, survivability and metabolic capacity in the host gut, the probiotic's host specificity, influence of feed processing (e.g., steam conditioning, pelleting) on the survivability of the probiotic in the final prepared diet, and differences in the experimental conditions—can play an important role in the effective responses observed following administration of probiotics (Taherpour et al. 2009). Use of probiotics sometimes incurs adverse effects especially when they competitively exclude indigenous beneficial microflora (Edens 2003). Probiotics often cause a transitory alteration in the indigenous gut microflora especially when large numbers of probiotic bacteria are introduced (Edens and Pierce 2010).

7.8   Future Directions

A comprehensive knowledge base is needed regarding the metabolites responsible for the effect of probiotics on host immune systems responding to the pathogenic bacteria. Considerable work remains to determine the mechanisms of action and optimum dose of any given probiotic. This includes not only elucidating the mechanism of action but developing an understanding of interactions in the host and the host's responsiveness to the probiotic. Genetic evaluation of probiotic and gut microbiota would help in selecting an appropriate probiotic supplement. Application of molecular approaches to identify/evaluate the microbial communities and their growth requirements is likely to divulge new microbial responses that can benefit the host (Ricke and Pillai 1999). Applying modern analytical techniques can be of great value in understanding the bacteria–diet interactions and the role of the various probiotic bacteria on animal health. These technological approaches would allow the advancement of therapeutic treatments in poultry management system and enhance the birds' nutrition through modified feed formulation to optimize growth and gut health. Identification tools based on molecular methods utilizing total bacterial DNA- or RNA-targeted probes and development of profiling tools such as DGGE,% G+C, gene amplification protocols, and mRNA analysis can further increase the ability to enssure validation before, during, and after application (Ricke and Pillai 1999; Hanning and Ricke 2011).

A combination of probiotics and naturally occurring components such as prebiotics, nonspecific substrates, plant extracts, and microbial metabolites that act synergistically to improve host health would be appealing and may yield a new dimension in using probiotics in the sphere of safe food practices. The beneficial effects of probiotics can be further enhanced by selecting more efficient strains or combinations of microorganism, gene manipulation, combination of probiotics and naturally occurring synergistically acting compounds such as prebiotics (Bomba et al. 2006). Synbiotics are nutritional supplements that contain a mixture of prebiotics and probiotics "that act synergistically and deliver beneficial effects to the host by improving the survival and implantation of live microbial dietary supplements in the GI tract" (Gibson and Roberfroid 1995). Adding prebiotics to animal feed would further increase the efficiency of probiotic culture preparations by improving the survival of probiotic bacteria through the upper GI tract, thereby inducing beneficial effects (Roberfroid 1998; Suskovic et al. 2001).

Most of the earlier studies were concerned with beneficial effects of probiotics, but it has been difficult to interpret their findings for one or more of the following reasons: no statistical interpretation of the experimental results; poor experimental protocols; and undetermined validity and viability of the probiotic strain (Simon et al. 2001). Thus, a comprehensive, clearly defined experimental protocol with valid statistical analysis should be undertaken for better application of the results in future research studies.

7.9 Conclusions

The major focus of this review was a summation of probiotic beneficial effects and their impact in poultry preharvest food safety based on changes in gut dynamics. These changes may include immune system stimulation and modulating the intestinal architecture with metabolic and physiological adjustments. A critical understanding of the interrelationship of GI physiology, microbiology, and their effect on the host immune system is crucial when selecting probiotics. Dietary supplementation of probiotics during poultry production has reduced the potential use of antibiotics and other growth promoters and therefore could be viewed as potentially safe for growth promotion. Furthermore, probiotics have the potential to improve preharvest food safety by reducing the enteric pathogen load. Nonetheless, none of these alternative strategies/products is sufficient to control the impact of food-borne pathogens; nor can they be effective under a wide variety of conditions unless more is understood about the specific mechanisms and their respective relations with the avian host.

References

Alkhalf, A., M. Alhaj, and I. Al-Homidan. 2010. Influence of probiotic supplementation on immune response of broiler chicks. *Egyptian Poultry Science* 30: 271–280.

Altekruse, S.F., N.J. Stern, P.I. Fields, and D.L. Swedlow. 1999. *Campylobacter jejuni* – An emerging food-borne pathogen. *Emerging Infectious Diseases* 5: 28–35.

Apajalahti, J., and M. Bedford. 2000. Impact of dietary and environmental factors on microbial communities of the avian GI tract. http://www.poultry-health.com/fora/inthelth/apabed01.htm. Accessed Dec 2010.

Apajalahti, J.H.A., H. Kettunen, M.R. Bedford, and W.E. Holben. 2001. Percent G+C profiling accurately reveals diet-related differences in the gastrointestinal microbial community of broiler chickens. *Applied and Environmental Microbiology* 67: 5656–5667.

Apajalahti, J.H., H. Kettunen, A. Kettunen, W.E. Holben, P.H. Nurminen, N. Rautonen, and M. Mutanen. 2002. Culture-independent microbial community analysis reveals that inulin in the diet primarily affects previously unknown bacteria in the mouse cecum. *Applied and Environmental Microbiology* 68: 4986–4995.

Apajalahti, J.H., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *World's Poultry Science Journal* 60: 223–232.

Appleby, M.C., J.A. Mench, and B.O. Hughes. 2004. *Poultry behavior and welfare*. Oxfordshire: CABI.

Ayasan, T., S. Yurtseven, M. Baylanm, and S. Canogullari. 2005. The effects of dietary *Yucca schidigera* on egg yield parameters and egg shell quality of laying Japanese quails (*Coturnix coturnix japonica*). *International Journal of Poultry Science* 4: 159–162.

Ayasan, T., and F. Okan. 2006. Determination of threonine requirements of female broiler chicks in starter period. *Journal of Faculty of Agriculture*, Kyushu University 21: 41–48.

Bailey, J.S. 1988. Integrated colonization control of *Salmonella* in poultry. *Poultry Science* 67: 928–932.

Barnes, E.M. 1972. The avian intestinal flora with particular reference to the possible ecological significance of the cecal anaerobic bacteria. *American Journal of Clinical Nutrition* 25: 1475–1479.

Barnes, E.M., G.C. Mead, D.A. Barnum, and E.G. Harry. 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. *British Poultry Science* 13: 311–326.

Bomba, A., R. Nemcova, S. Gancarcikova, R. Herich, P. Guba, and D. Mudronova. 2002. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructooligosaccharides and polyunsaturated fatty acids. *British Journal of Nutrition Supplement* 1: 95–99.

Bomba, A., Z. Jonecova, J. Koscova, R. Nemcova, S. Gancarcıkova, D. Mudronova, L. Scirankova, V. Buleca, G. Lazar, J. Posivak, R. Kastel, and M. Marekova. 2006. The improvement of probiotics efficacy by synergistically acting components of natural origin: A review. *Biologia* 61: 712–729.

Briandet, R., T. Meylheuc, C. Maher, and M.N. Bellon-Fontaine. 1999. *Listeria monocytogenes* Scott A: Cell surface charge, hydrophobicity and electron donor and acceptor characteristics under different environmental growth conditions. *Applied and Environmental Microbiology* 65: 5328–5333.

Bryan, F.L. 2001. Reflections on a career in public health: Evolving food-borne pathogens, environmental health, and food safety programs. *Journal of Environmental Health* 65: 14–24.

Burton, J., J. McCormick, P. Cadieux, and G. Reid. 2003. Digoxigenin-labelled peptide nucleic acid to detect *Lactobacilli* PCR amplicons immobilized on membranes from denaturing gradient gel electrophoresis. *Letters in Applied Microbiology* 36: 145–149.

Buts, J., N. De Keyser, C. Stilmant, E. Sokal, and S. Marandi. 2002. *Saccharomyces boulardii* enhances N-terminal peptide hydrolysis in suckling rat small intestine by endoluminal release of a zinc-binding metalloprotease. *Pediatric Research* 51: 528–534.

Byrd, J.A., D.E. Corrier, M.E. Hume, R.H. Bailey, L.H. Stanker, and B.M. Hargis. 1998. Effect of feed withdrawal on the incidence of Campylobacter in crops of preharvest broiler chickens. *Avian Diseases* 42: 802–806.

Caballero-Franco, C., K. Keller, C. De Simone, and K. Chadee. 2007. The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *American Journal of Physiology, Gastrointestinal and Liver Physiology* 292: 315–322.

Catala-Gregori, P., V. Garcia, J. Madrid, J. Orengo, and F. Hernandez. 2007. Response of broilers to feeding low-calcium and total phosphorus wheat-soybean based diets plus phytase: Performance, digestibility, mineral retention and tibiotarsus mineralization. *Canadian Journal of Animal Science* 87: 563–569.

Centers for Disease and Control and Prevention (CDC). 2009. Food-borne illness. http://topnews. us/content/24800-cdc-food-borne-illnesses-high-2008-2004. Accessed Oct 23, 2010.

Chandrasekhar, S. 2009. Modern dietary approach to prevent enteric disorders in commercial poultry production. Neospark drugs and chemicals. http://en.engormix.com/MA-poultry-industry/health/articles/modern-dietary-approach-prevent-t1223/165-p0.htm. Acessed on Nov 15, 2011.

Chichlowski, M., J. Croom, B.W. McBride, G.B. Havenstein, and M.D. Koci. 2007a. Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. *International Journal of Poultry Science* 6: 694–704.

Chichlowski, M., W.J. Croom, B.W. McBride, R. Qiu Chiang, L.R. Daniel, et al. 2007b. Micro-architecture and spatial relationship between bacteria and ileal, cecal and colonic epithelium in chicks fed a direct-fed microbial and salinomycin. *Poultry Science* 86: 1121–1132.

Choct, M. 2009. Managing gut health through nutrition. *British Poultry Science* 50: 9–15.

Choct, M., R.J. Hughes, J. Wang, M.R. Bedford, A.J. Morgan, and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* 37: 609–621.

Christensen, H.R., H. Frokiaer, and J.J. Pestka. 2002. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. *Journal of Immunology* 168: 171–178.

Coloe, P.J., J.J. Bagust, and L. Ireland. 1984. Development of the normal gastrointestinal microflora of specific pathogen-free chicken. *The Journal of Hygiene* 92: 79–87.

Conway, P.L., S.L. Gorbach, and B.R. Goldin. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *Journal of Dairy Science* 70: 1–12.

Corrier, D.E., D.J. Nisbet, C.M. Scanlan, A.G. Hollister, D.J. Caldwell, L.A. Thomas, B.M. Hargis, T. Tompkins, and J.R. DeLoach. 1995a. Treatment of commercial broiler chickens with a characterized culture of cecal bacteria to reduce Salmonellae colonization. *Poultry Science* 74: 1093–1101.

Corrier, D.E., D.J. Nisbet, C.M. Scanlan, A.G. Hollister, and J.R. DeLoach. 1995b. Control of *Salmonella typhimurium* colonization in broiler chicks with a continuous flow characterized mixed culture of cecal bacteria. *Poultry Science* 74: 916–924.

Corrier, D.E., D.J. Nisbet, B.M. Hargis, P.S. Holt, and J.R. DeLoach. 1997. Provision of lactose to molting hens enhances resistance to *Salmonella enteritidis* colonization. *Journal of Food Protection* 60: 10–15.

Corrier, D.E., J.A. Byrd, B.M. Hargis, M.E. Hume, R.H. Bailey, and L.H. Stanker. 1999. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. *Poultry Science* 78: 45–49.

Corry, J.E.L., V.M. Allen, W.R. Hudson, M.F. Breslin, and R.H. Davies. 2002. Sources of *Salmonella* on broiler carcasses during transportation and processing: Modes of contamination and methods of control. *Journal of Applied Microbiology* 92: 424–432.

Cressman, M.D. 2009. A molecular approach to understanding the interrelation between the microbiomes in the litter and intestines of commercial broiler chickens. MS Thesis. Columbus: Ohio State University.

Cummings, J.H., and G.T. Macfarlane. 1997. Role of intestinal bacteria in nutrient metabolism. *Journal of Parenteral and Enteral Nutrition* 21: 357–365.

Czinn, S.J., and S.S. Blanchard. 2009. Probiotics in foods and supplements. In *Nutrition and health: Probiotics in pediatric medicine*, ed. S. Michail and P.M. Sherman, 299–306. Totowa: Humana Press.

Dalloul, R.A., H.S. Lillehoj, T.A. Shellem, and J.A. Doerr. 2003a. Intestinal immunomodulation by vitamin A deficiency and lactobacillus-based probiotic in *Eimeria acervulina* infected broiler chickens. *Avian Diseases* 47: 1313–1320.

Dalloul, R.A., H.S. Lillehoj, T.A. Shellem, and J.A. Doerr. 2003b. Enhanced mucosal immunity against *Eimeria acervulina* in broilers fed a *Lactobacillus* based probiotic. *Poultry Science* 82: 62–66.

Dänicke, S., W. Vahjen, O. Simon, and H. Jeroch. 1999. Effects of dietary fat type and xylanase supplementation to rye-based broiler diets on selected bacterial groups adhering to the intestinal epithelium, on transit time of feed, and on nutrient digestibility. *Poultry Science* 78: 1292–1299.

Dash, S.K. 2009. Selection criteria for probiotics. In *Presented at XXXVII Dairy industry conference*. Panjim: Kala Academy.

Dizaji, S.B., and R. Piromohammadi. 2009. Effect of *Saccharomyces cerevisiae* and Bioplus 2B on performance of laying hens. *International Journal of Agriculture and Biology* 11: 495–497.

Doyle, M.P., and M.C. Erickson. 2006. Emerging microbiological food safety issues related to meat. *Meat Science* 74: 98–112.

Duggan, C., J. Gannon, and W.A. Walker. 2002. Protective nutrients and functional foods for the gastrointestinal tract. *American Journal of Clinical Nutrition* 75: 789–808.

Dunkley, C.S., J.L. McReynolds, K.D. Dunkley, L.F. Kubena, D.J. Nisbet, and S.C. Ricke. 2007a. Molting in *Salmonella* Enteritidis-challenged laying hens fed alfalfa crumbles. III. Blood plasma metabolite response. *Poultry Science* 86: 2492–2501.

Dunkley, C.S., J.L. McReynolds, K.D. Dunkley, L.N. Njongmeta, L.R. Berghman, L.F. Kubena, D.J. Nisbet, and S.C. Ricke. 2007b. Molting in *Salmonella* Enteritidis challenged laying hens fed alfalfa crumbles. IV. Immune and stress protein response. *Poultry Science* 86: 2502–2508.

Dunkley, K.D., J.L. McReynolds, M.E. Hume, C.S. Dunkley, T.R. Callaway, L.F. Kubena, D.J. Nisbet, and S.C. Ricke. 2007c. Molting in *Salmonella* Enteritidis challenged laying hens fed alfalfa crumbles. I. Salmonella Enteritidis colonization and virulence gene *hilA* response. *Poultry Science* 86: 1633–1639.

Dunkley, K.D., J.L. McReynolds, M.E. Hume, C.S. Dunkley, T.R. Callaway, L.F. Kubena, D.J. Nisbet, and S.C. Ricke. 2007d. Molting in *Salmonella* Enteritidis challenged laying hens fed alfalfa crumbles.II. Fermentation and microbial ecology response. *Poultry Science* 86: 2101–2109.

Dunkley, K.D., T.R. Callaway, V.I. Chalova, J.L. McReynolds, M.E. Hume, C.S. Dunkley, L.F. Kubena, D.J. Nisbet, et al. 2009. Food-borne *Salmonella* ecology in the avian gastrointestinal tract. *Anaerobe* 15: 26–35.

Durant, J.A., D.E. Corrier, J.A. Byrd, L.H. Stanker, and S.C. Ricke. 1999. Feed deprivation affects crop environment and modulates *Salmonella enteritidis* colonization and invasion of Leghorn hens. *Applied and Environmental Microbiology* 65: 1919–1923.

Durant, J.A., D.E. Corrier, L.H. Stanker, and S.C. Ricke. 2000. Expression of the *hilA Salmonella typhimurium* gene in a poultry *Salm. enteritidis* isolate in response to lactate and nutrients. *Journal of Applied Microbiology* 89: 63–69.

EC. 2001. Commission of the European Communities, Commission Recommendation, 2001/459/EC. *Official Journal of European Union* L161: 42–44.

EC. 2003. Commission of the European Communities, Commission Regulation (EC) No. 1831/2003. *Official Journal of European Union* L268: 29–33.

Edens, F.W. 2003. An alternative for antibiotic use in poultry: Probiotics. *Revista Brasileira de Ciência Avícola* 5: 75–97.

Edens, F., and J. Pierce. 2010. Nutrigenomics: Implications for prebiotics and intestinal health. Alltech technical symposium, Arkansas nutrition conference, Rogers.

EL-Banna, H.A., H.Y. EL-Zorba, T.A. Attia, and A.A. Elatif. 2010. Effect of probiotic, prebiotic and synbiotic on broiler performance. *World Applied Science Journal* 11: 388–393.

FAO/WHO. 2002. *Guidelines for the evaluation of probiotics in food*. London: FAO/WHO.

Farnell, M.B., A.M. Donoghue, F.S. de Los Santos, P.J. Blore, B.M. Hargis, G. Tellez, and D.J. Donoghue. 2006. Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. *Poultry Science* 85: 1900–1906.

Fooks, L., and G. Gibson. 2002. Probiotics as modulators of the gut flora. *British Journal of Nutrition* 88: 39–49.

FSIS/USDA. 2010. Pre-harvest management controls and intervention options for reducing *Escherichia coli* O157:H7 shedding in cattle. www.fsis.usda.gov/PDF/Reducing_Ecoli_Shedding_In_Cattle_0510.pdf.

Fuller, R. 1973. Ecological studies on the *Lactobacillus* flora associated with the crop epithelium of the fowl. *Journal of Applied Bacteriology* 36: 131–139.

Fuller, R. 1975. Nature of the determinant responsible for the adhesion of lactobacilli to chicken crop epithelial cells. *Journal of General Microbiology* 87: 245–250.

Fuller, R. 1977. The importance of *Lactobacilli* in maintaining normal microbial balance in the crop. *British Poultry Science* 18: 85–94.

Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology* 66: 365–378.

Fuller, R. 2001. The chicken gut microflora and probiotic supplements (review). *Poultry Science* 38: 189–196.

Gardiner, G., C. Heinemann, D. Beuerman, A.W. Bruce, and G. Reid. 2002. Persistence of *Lactobacillus fermentum* RC-14 and *L. rhamnosus* GR-1, but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA (RAPD). *Clinical and Diagnostic Laboratory Immunology* 9: 92–96.

Gaskins, H.R., C.C. Collier, and D.B. Anderson. 2002. Antibiotics as growth promotants: Mode of action. Proceedings of the Conference on Antibiotic use in Animal Agriculture. (Ed. RI Mackie). *Animal Biotechnology* 13: 29–42.

Gebreyes, W.A., P.B. Bahnson, J.A. Funk, J. McKean, and P. Patchanee. 2008. Seroprevalence of *Trichinella*, *Toxoplasma*, and *Salmonella* in antimicrobial-free and conventional swine production systems. *Food-borne Pathogens Disease* 5: 199–203.

Ghafoor, A., S. Naseem, M. Younus, and J. Nazir. 2005. Immunomodulatory effects of multistrain probiotics (protexin™) on broiler chicken vaccinated against avian influenza virus (H9). *International Journal of Poultry Science* 4: 777–780.

Gibson, G.R. 1999. Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *Journal of Nutrition* 129: 1438–1441.

Gibson, G.R., and R. Fuller. 2000. Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. *Journal of Nutrition* 130: 391–395.

Gibson, G.R., and M.B. Roberfroid. 1995. Dietary modulation of the human colonic microflora: Introducing the concept of prebiotics. *Journal of Nutrition* 125: 1401–1412.

Gibson, G.R., J.M. Saavedra, and S. MacFarlane. 1997. Probiotics and intestinal microflora. In *Probiotics: Therapeutic and other beneficial effects*, ed. R. Fuller, 10–39. London: Chapman & Hall.

Gong, J., R.J. Forster, H. Yu, J.R. Chambers, P.M. Sabour, R. Wheatcroft, and S. Chen. 2002. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken ceca and comparison with bacteria in the cecal lumen. *FEMS Microbiology Letters* 208: 1–7.

Goren, E., W.A. de Jong, P. Doorenbal, J.P. Koopman, and H.M. Kennis. 1984. Protection of chicks against *Salmonella infantis* infection induced by strict anaerobically cultured intestinal microflora. *Veterinary Quarterly* 6: 22–26.

Guillot, J.F. 1998. Les probiotiques en alimentation animale. *Cahiers Agricultures* 7: 49–54.

Guillot, J.F. 2009. Consequences of probiotics release in the intestine of animals. Universite de Tours-IUT 29 rue de Pont-Vollant, 37082 Tours Cedex 2, France: 17–21.

Ha, S.D., S.C. Ricke, D.J. Nisbet, D.E. Corrier, and J.R. DeLoach. 1994. Serine utilization as a potential competition mechanism between *Salmonella* and a chicken cecal bacterium. *Journal of Food Protection* 57: 1074–1079.

Ha, S.D., S.C. Ricke, D.J. Nisbet, D.E. Corrier, and J.R. DeLoach. 1995. Comparison of *Salmonella typhimurium* and selected facultative chicken bacteria survivability after specific amino acid limited batch growth. *Journal of Food Protection* 58: 1335–1339.

Haghighi, H.R., J.H. Gong, C.L. Gyles, M.A. Hayes, et al. 2006. Probiotics stimulate production of natural antibodies in chickens. *Clinical and Vaccine Immunology* 13: 975–980.

Hanning, I., and S.C. Ricke. 2011. Prescreening methods of microbial populations for the assessment of sequencing potential. In *Methods in molecular microbiology 733 – High-throughput next generation sequencing: Methods and applications*, ed. Y.M. Kwon and S.C. Ricke, 159–170. New York: Springer Protocols, Humana Press.

Havenaar, R., and M.J.H. Huis In't Veld. 1992. Probiotics: A general view. In *Lactic acid bacteria in health and disease*, vol. 1, ed. B.J.B. Wood, 271–281. Amsterdam: Elsevier Applied Science Publishers.

Havenaar, R., and S. Spanhaak. 1994. Probiotics from an immunological point of view. *Current Opinion in Biotechnology* 5: 320–325.

Heczko, U., A. Abe, and B.B. Finlay. 2000. Segmented filamentous bacteria prevent colonization of enteropathogenic *Escherichia coli* O103 in rabbits. *Journal of Infectious Diseases* 181: 1027–1033.

Henriksson, A., R. Szewzyk, and P.L. Conway. 1991. Characteristics of the adhesive determinants of *Lactobacillus fermentum* 104. *Applied and Environmental Microbiology* 57: 499–502.

Heuer, O.E., K. Pedersen, J.S. Andersen, and M. Madsen. 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. *Letters in Applied Microbiology* 33: 269–274.

Higgins, S.E., A. Torres-Rodriguez, 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. *Journal of Applied Poultry Research* 14: 345–348.

Higgins, J.P., S.E. Higgins, J.L. Vicente, A.D. Wolfenden, G. Tellez, and B.M. Hargis. 2007a. Temporal effects of lactic acid bacteria probiotic on *Salmonella* in neonatal broilers. *Poultry Science* 86: 1662–1666.

Higgins, S.E., G.F. Erf, J.P. Higgins, S.N. Henderson, A.D. Wolfenden, G. Gaona-Ramirez, and B.M. Hargis. 2007b. Effect of probiotic treatment in broiler chicks on intestinal macrophage numbers and phagocytosis of *Salmonella* Enteritidis by abdominal exudate cells. *Poultry Science* 86: 2315–2321.

Higgins, S.E., J.P. Higgins, A.D. Wolfenden, S.N. Henderson, A. Torres-Rodriguez, G. Tellez, and B.M. Hargis. 2008. Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella* Enteritidis in neonatal broiler chicks. *Poultry Science* 87: 27–31.

Hinton Jr., A., D.E. Corrier, and J.R. DeLoach. 1992. In vitro inhibition of *Salmonella typhimurium* and *Escherichia coli* O157:H7 by an anaerobic Gram-positive coccus isolated from the cecal contents of adult chickens. *Journal of Food Protection* 55: 162–166.

Hinton, A., R.J. Buhr, and K.D. Ingram. 2000. Reduction of *Salmonella* in the crop of broiler chickens subjected to feed withdrawal. *Poultry Science* 79: 1566–1570.

Hofacre, C.L., R. Froyman, B. Gautrias, B. George, M.A. Goodwin, and J. Brown. 1998. Use of Aviguard and other intestinal bioproducts in experimental *Clostridium perfringens* associated necrotizing enteritis in broiler chickens. *Avian Diseases* 42: 579–584.

Hofacre, C.L., T. Beacorn, S. Collet, and G.J. Mathis. 2003. Using competitive exclusion, mannanoligosaccharide and other intestinal products to control necrotic enteritis. *Journal of Applied Poultry Research* 12: 60–64.

Holben, W.E., L.K. Sarkilahti, P. Williams, M. Saarinen, and J.H.A. Apajalahti. 2002. Phylogenetic analysis of intestinal microflora indicates a novel *Mycoplasma* phylotype in farmed and wild salmon. *Microbial Ecology* 44: 175–185.

Holt, P.S., N.P. Macri, and R.E. Porter Jr. 1995. Microbiological analysis of the early Salmonella enteritidis infection in molted and unmolted hens. *Avian Diseases* 39: 55–63.

Huang, Y., and M.C. Adams. 2003. An in vitro model for investigating intestinal adhesion of potential dairy propionibacteria probiotic strains using cell line C2BBe1. *Letters in Applied Microbiology* 36: 213–216.

Jadamus, A., W. Vahjen, and I. Kühn. 2000. The effects of the probiotic toyocerin in fattening poultry. *Lohmann information* 23: 3–6.

Jin, L.Z., Y.W. Ho, N. Abdullah, and S. Jalaludin. 1996. Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chickens. *Letters in Applied Microbiology* 23: 67–71.

Johnson, M.E., and J.L. Steele. 2007. Chapter 35. Fermented dairy products. In *Food Microbiology-Fundamentals and Frontiers*, 3rd ed, ed. M.P. Doyle and L.R. Beuchat, 767–781. Washington, DC: American Society for Microbiology.

Kalavathy, R., N. Abdullah, S. Jalaludin, and Y.W. Ho. 2003. Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *British Poultry Science* 44: 139–144.

Khambualai, O., K. Yamauchi, J. Ruttanavut, T. Incharoen, and J. Kashimura. 2010. Effect of sugar cane extract, commercial probiotic and their mixture on growth performance and intestinal histology in broiler chickens. *American Journal of Animal and Veterinary Sciences* 5: 132–138.

Knarreborg, A., M.A. Simon, R.M. Engberg, B.B. Jensen, and G.W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. *Applied and Environmental Microbiology* 68: 5918–5924.

Kohler, H., S.P. Rodrigues, A.T. Maurelli, and B.A. McCormick. 2002. Inhibition of *Salmonella typhimurium* enteropathogenicity by piperidine, a metabolite of the polyamine cadaverine. *Journal of Infectious Diseases* 186: 1122–1130.

La Ragione, R.M., and M.J. Woodward. 2003. Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype *enteritidis* and *Clostridium perfringens* in young chickens. *Veterinary Microbiology* 94: 245–256.

Lammers, K.M., P. Brigidi, B. Vitali, P. Gionchetti, F. Rizzello, E. Caramelli, D. Matteuzzi, and M. Campieri. 2003. Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. *FEMS Immunology and Medical Microbiology* 38: 165–172.

Lan, P.T., T.L. Binh, and Y. Benno. 2003. Impact of two probiotics *Lactobacillus* strains feeding on fecal *Lactobacilli* and weight gains in chickens. *Journal of General and Applied Microbiology* 49: 29–36.

Lessard, M., M. Dupuis, N. Gagnon, É. Nadeau, J.J. Matte, J. Goulet, and J.M. Fairbrother. 2009. Administration of *Pediococcus acidilactici* or *Saccharomyces cerevisiae boulardii* modulates development of porcine mucosal immunity and reduces intestinal bacterial translocation after *Escherichia coli* challenge. *Journal of Animal Science* 87: 922–934.

Lloyd, A.B., R.B. Cumming, and R.D. Kent. 1974. Competitive exclusion as exemplified by *Salmonella typhimurium*. In *Proceedings Australian poultry science convention*, 185–186. Hobart.

Loneragan, G.H., and M.M. Brashears. 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Science* 71: 72–78.

Maassen, C.B., C. van Holten-Neelen, F. Balk, M.J. den Bak-Glashouwer, R.J. Leer, J.D. Laman, W.J. Boersma, and E. Claassen. 2000. Strain dependent induction of cytokine profiles in the gut by orally administered *Lactobacillus* strains. *Vaccine* 18: 613–623.

Madsen, K., A. Cornish, P. Soper, C. McKaigney, H. Jijon, C. Yachimec, J. Doyle, L. Jewell, and C. DeSimone. 2001. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 121: 580–591.

Mahdavi, A.H., H.R. Rahmani, and J. Pourreza. 2005. Effect of probiotic supplements on egg quality and laying hen's performance. *International Journal of Poultry Science* 4: 488–492.

Maidak, B.L., J.R. Cole, C.T. Parker Jr., G.M. Garrity, et al. 1999. A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Research* 27: 171–173.

Marteau, P. 2002. Probiotics in clinical conditions. *Clinical Reviews in Allergy and Immunology* 22: 255–273.

Marteau, P., P. Seksik, P. Lepage, and J. Dore. 2004. Cellular and physiological effects of probiotics and prebiotics. *Mini Reviews in Medicinal Chemistry* 4: 889–896.

Martin, C., E. Dunlap, S. Caldwell, E. Barnhart, N. Keith, and J.R. DeLoach. 2000. Drinking water delivery of a defined competitive exclusion culture (Preempt™) in 1-day-old broiler chicks. *Journal of Applied Poultry Research* 9: 88–91.

Mason, C.K., M.A. Collins, and K. Thompson. 2005. Modified electroporation protocol for *Lactobacilli* isolated from the chicken crop facilitates transformation and the use of a genetic tool. *Journal of Microbiological Methods* 60: 353–363.

Mathur, S., and R. Singh. 2005. Antibiotic resistance in food lactic acid bacteria – A review. *International Journal of Food Microbiology* 105: 281–295.

May, J.D., and B.D. Lott. 1990. Managing feed withdrawal. *Poultry Digest* 49: 48–50.

McReynolds, J., C. Waneck, J. Byrd, K. Genovese, S. Duke, and D. Nisbet. 2009. Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. *Poultry Science* 88: 2075–2080.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, et al. 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases* 5: 607–625.

Meghrous, J., P. Euloge, A.M. Junelles, J. Ballongue, and H. Petitdemange. 1990. Screening of *Bifidobacterium* strains for bacteriocin production. *Biotechnology Letters* 12: 575–580.

Metchnikoff, E. 1907. Essais optimistes. Paris. The prolongation of life. Optimistic studies. (trans: Chalmers Mitchell, P). London.

Metchnikoff, E. 1908. *Prolongation of life*, 161–183. New York: GP Putnam's & Sons.

Miller, W.G., and R.E. Mandrell. 2005. Prevalence of *Campylobacter* in the food and water supply: Incidence, outbreaks, isolation and detection. In *Campylobacter: Molecular and cellular biology*, ed. J.M. Ketley and M.E. Konkel, 101–163. Norfolk: Horizon Scientific Press.

Montalto, M., N. Maggiano, R. Ricci, V. Curigliano, L. Santoro, F. Di Nicuolo, F.M. Vecchio, A. Gasbarrini, and G. Gasbarrini. 2004. *Lactobacillus acidophilus* protects tight junctions from aspirin damage in HT-29 cells. *Digestion* 69: 225–228.

Moore, R.W., S.Y. Park, L.F. Kubena, J.A. Byrd, J.L. McReynolds, M.R. Burnham, M.E. Hume, S.G. Birkhold, D.J. Nisbet, and S.C. Ricke. 2004. Comparison of zinc acetate and propionate addition on gastrointestinal tract fermentation and susceptibility of laying hens to *Salmonella enteritidis* during forced molt. *Poultry Science* 83: 1276–1286.

Mountzouris, K.C., P. Tsistsikos, E. Kalamara, S. Nitsh, G. Schatzmayr, and K. Fegeros. 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science* 86: 309–317.

Mountzouris, K.C., P.T. Sitrsikos, I. Palamidi, A. Arvaniti, M. Mohnl, G. Schatzmayr, and K. Fegeros. 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science* 89: 58–67.

Nakamura, A., Y. Ota, A. Mizukami, et al. 2002. Evaluation of Aviguard, a commercial competitive exclusion product for efficacy and after-effect on the antibody response of chicks to *Salmonella*. *Poultry Science* 81: 1653–1660.

Newell, D.G., and H.C. Davison. 2003. *Campylobacter*: Control and prevention. In *Microbial food safety in animal agriculture*, ed. M.E. Torrence and R.E. Isaacson, 211–220. Ames: Iowa State University Press.

Newell, D.G., and C. Fearnly. 2003. Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology* 69: 4343–4351.

Nijdam, E., E. Lambooij, M.J. Nabuurs, E. Decuypere, and J.A. Stegeman. 2006. Influences of feeding conventional and semisynthetic diets and transport of broilers on weight gain, digestive tract mass, and plasma hormone and metabolite concentrations. *Poultry Science* 85: 1652–1659.

Nisbet, D. 2002. Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine. *Antonie Van Leeuwenhoek* 81: 481–486.

Nisbet, D.J., D.E. Corrier, and J.R. DeLoach. 1993. Effect of mixed cecal microflora maintained in continuous culture and of dietary lactose on Salmonella typhimurium colonization in broiler chicks. *Avian Diseases* 37: 528–535.

Nisbet, D.J., S.C. Ricke, C.M. Scanlan, D.E. Corrier, A.G. Hollister, and J.R. DeLoach. 1994. Inoculation of broiler chicks with a continuous-flow derived bacterial culture facilitates early cecal bacterial colonization and increases resistance to *Salmonella typhimurium*. *Journal of Food Protection* 57: 12–25.

Nisbet, D.J., D.E. Corrier, S.C. Ricke, M.E. Hume, J.A. Byrd, and J.R. De Loach. 1996a. Maintenance of the biological efficacy in chicks of a cecal competitive-exclusion culture against *Salmonella* by continuous-flow fermentation. *Journal of Food Protection* 59: 1279–1283.

Nisbet, D.J., D.E. Corrier, S.C. Ricke, M.E. Hume, J.A. Byrd, and J.R. De Loach. 1996b. Cecal propionic acid as a biological indicator of the early establishment of a microbial ecosystem inhibitory to *Salmonella* in chicks. *Anaerobe* 2: 345–350.

Norberg, L.M., J.L. McReynolds, W.K. Kim, V.I. Chalova, D.J. Nisbet, and S.C. Ricke. 2010. Colonization and pathogenesis of food-borne *Salmonella* in egg-laying hens. In *Perspectives on food-safety issues of animal-derived foods*, ed. S.C. Ricke and F.T. Jones, 63–84. Fayetteville: University of Arkansas Press.

Northcutt, J.K., R.J. Buhr, M.E. Berrang, and D.L. Fletcher. 2003. Effects of replacement finisher feed and length of feed withdrawal on broiler carcass yield and bacteria recovery. *Poultry Science* 82: 1820–1824.

Nurmi, E., and M. Rantala. 1973. New aspects of *Salmonella* infection in broiler production. *Nature* 241: 210–211.

Nurmi, E., L. Nuotio, and C. Schncitz. 1992. The competitive exclusion concept: Development and future. *International Journal of Food Microbiology* 15: 237–240.

Ochi, Y., T. Mitsuoka, and T. Sega. 1964. Untersuchungen über die Darmflora des Huhnes, III Mitteilung: Die Entwicklung der Darmflora von Küken bis zum Huhn. *Zentbl. Bakt, ParasitKde* 193: 80–85. Abt 1.

Oliver, S.P., D.A. Patel, T.R. Callaway, and M.E. Torrence. 2009. Developments and future outlook for preharvest food safety. *Journal of Animal Science* 87: 419–437.

Oyofo, B.A., J.R. De Loach, D.E. Corrier, J.O. Norman, R.L. Ziprin, and H.H. Mollenhauer. 1989a. Prevention of *Salmonella typhimurium* colonization of broilers with D-mannose. *Poultry Science* 68: 1357–1360.

Oyofo, B.A., J.R. De Loach, D.E. Corrier, J.O. Norman, R.L. Ziprin, and H. Mollenhaur. 1989b. Effect of carbohydrates on Salmonella typhimurium colonization in broiler chickens. *Avian Diseases* 33: 531–534.

Oyofo, B.A., R.E. Droleskey, J.O. Norman, H.H. Mollenhauer, R.L. Ziprin, D.E. Corrier, and J.R. De Loach. 1989c. Inhibition by mannose of in vitro colonization of chicken small intestine by *Salmonella typhimurium*. *Poultry Science* 68: 1351–1356.

Park, S.Y., C.L. Woodward, L.F. Kubena, D.J. Nisbet, S.G. Birkhold, and S.C. Ricke. 2008. Environmental dissemination of food-borne salmonella in preharvest poultry production: Reservoirs, critical factors, and research strategies. *Critical Reviews in Environmental Science and Technology* 38: 73–111.

Parker, R.B. 1974. Probiotics, the other half of the antibiotic story. *Animal Nutrition and Health* 29: 4–8.

Patterson, J.A., and K. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. *Poultry Science* 82: 627–631.

Pivnick, H., and E. Nurmi. 1982. The Nurmi concept and its role in the control of *Salmonella* in poultry. In *Developments in food microbiology*, vol. 1, ed. R. Davies, 41–70. Barking: Applied Science Publishers Ltd.

Pour, J.B., and H. Kermanshahi. 2010. Effects of cecal cultures and a commercial probiotic (PremaLac®) on performance and serum lipids of broiler chickens. *Journal of Animal and Veterinary Advances* 9: 1506–1509.

Przyrembel, H. 2001. Consideration of possible legislation within existing regulatory frameworks. *American Journal of Clinical Nutrition* 73(suppl.): 471–475.

Rahimi, M. 2009. Effects of probiotic supplementation on performance and humoral immune response of broiler chickens. In *Book of proceedings, 2nd Mediterranean Summit of WPSA.*, 67–69.

Ramirez, G.A., L.L. Sarlin, D.J. Caldwell, C.R. Yezak, M.E. Hume, D.E. Corrier, J.R. DeLoach, and B.M. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. *Poultry Science* 76: 654–656.

Rantala, M., and E. Nurmi. 1973. Prevention of the growth of *Salmonella infantis* in chickens by flora of the alimentary tract of chickens. *British Poultry Science* 14: 627–630.

Rasschaert, G., K. Houf, and L. DeZutter. 2006. Impact of the slaughter line contamination on the presence of Salmonella on broiler carcasses. *Journal of Applied Microbiology* 103: 333–341.

Rasschaert, G., K. Houf, C. Godard, C. Wildemauwe, M. Pastuszczak-Frak, and L. DeZutter. 2008. Contamination of carcasses with Salmonella during poultry slaughter. *Journal of Food Protection* 71: 146–152.

Rastall, R. 2004. Bacteria in the gut: Friends and foes and how to alter the balance. *Journal of Nutrition* 134: 2022–2026.

Reid, G. 2005. The importance of guidelines in the development and application of probiotics. *Current Pharmaceutical Design* 11: 11–16.

Reid, G., R.L. Cook, and A.W. Bruce. 1987. Examination of strains of *lactobacilli* for properties which may influence bacterial interference in the urinary tract. *Journal of Urology* 138: 330–335.

Reynolds, D. 1998. Proceedings of II Bayer European poultry symposium, 33–36. Cascais.

Ricke, S.C. 2003a. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science* 82: 632–639.

Ricke, S.C. 2003b. The gastrointestinal tract ecology of *Salmonella* Enteritidis colonization in molting hens. *Poultry Science* 82: 1003–1007.

Ricke, S.C., and F.T. Jones. 2010. *Perspectives on food safety issues of food animal-derived foods*. Fayetteville: University of Arkansas Press.

Ricke, S.C., and S.D. Pillai. 1999. Conventional and molecular methods for understanding probiotic bacteria functionality in gastrointestinal tracts. *Critical Reviews in Microbiology* 25: 19–38.

Ricke, S.C., C.L. Woodward, Y.M. Kwon, L.F. Kubena, and D.J. Nisbet. 2004. Limiting avian gastrointestinal tract *Salmonella* colonization by cecal anaerobic bacteria, and a potential role for methanogens. In *Preharvest and postharvest food safety: Contemporary issues and future directions*, ed. R.C. Beier, S.D. Pillai, T.D. Phillips, and R.L. Ziprin, 141–150. Ames: Blackwell Publ. Prof.

Ricke, S.C., I.B. Zabala-Diaz, and J.T. Keeton. 2007. Chapter 37. Fermented meat, poultry, and fish. In *Food microbiology-fundamentals and frontiers*, 3rd ed, ed. M.P. Doyle and L.R. Beuchat, 795–815. Washington, DC: American Society for Microbiology.

Roberfroid, M.B. 1998. Prebiotics and synbiotics: Concepts and nutritional properties. *British Journal of Nutrition* 80(Suppl. 2): S197–S202.

Roberfroid, M.B. 2000. Prebiotics and probiotics: Are they functional foods? *American Journal of Clinical Nutrition* 71: 1682–1687.

Saavedra, J. 1995. Microbes to fight microbes: A not so novel approach to controlling diarrheal disease. *Journal of Pediatric Gastroenterology and Nutrition* 21: 125–129.

Saavedra, J.M., N.A. Bauman, I. Oung, J.A. Perman, and R.H. Yolken. 1994. Feeding of *Bifidibacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 334: 1046–1049.

Šabatková, J., I. Kumprecht, P. Zobač, P. Suchý, and B. Čermák. 2008. The probiotic Bioplus 2B as an alternative to antibiotics in diets for broiler chickens. *Acta Veterinaria Brno* 77: 569–574.

Saengkerdsub, S., R.C. Anderson, H.H. Wilkinson, W.K. Kim, D.J. Nisbet, and S.C. Ricke. 2007a. Identification and quantification of methanogenic archaea in adult chicken ceca. *Applied and Environmental Microbiology* 73: 353–356.

Saengkerdsub, S., P. Herrera, L. Woodward, R.C. Anderson, D.J. Nisbet, and S.C. Ricke. 2007b. Detection of methane and quantification of methanogenic archaea in faeces from young broiler chickens using real-time PCR. *Letters in Applied Microbiology* 45: 629–634.

Salminen, S., E. Isolauri, and E. Salminen. 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. *Antonie Van Leeuwenhoek* 70: 347–358.

Schaafsma, S. 1996. State of art concerning probiotic strains in milk products. *IDF Nutr News Lett* 5: 23–24.

Schiffrin, E.J., F. Rochat, H. Link-Amster, J.M. Aeschlimann, and A. Donnet-Hughes. 1995. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *Journal of Dairy Science* 78: 491–497.

Schneitz, C. 2005. Competitive exclusion in poultry – 30 years of research. *Food Control* 16: 657–667.

Seo, K.H., P.S. Holt, and R.K. Gast. 2001. Comparison of *Salmonella* Enteritidis infection in hens molted via longterm withdrawal versus full-fed wheat middling. *Journal of Food Protection* 64: 1917–1921.

Servin, A.L., and M.H. Coconnier. 2003. Adhesion of probiotic strains to the intestinal enterocytes and interaction with pathogens. *Best Practice and Res Clin Gastroenterol* 17: 741–754.

Shakouri, M.D., P.A. Iji, L.L. Mikkelsen, and A.J. Cowieson. 2008. Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. *Journal of Animal Physiology and Animal Nutrition* 93: 647–658.

Shen, T.Y., H.L. Qin, Z.G. Gao, X.B. Fan, X.M. Hang, and Y.Q. Jiang. 2006. Influences of enteral nutrition combined with probiotics on gut microflora and barrier function of rats with abdominal infection. *World Journal of Gastroenterology* 12: 4352–4358.

Si, J.M., Y.C. Yu, Y.J. Fan, and S.J. Chen. 2004. Intestinal microecology and quality of life in irritable bowel syndrome patients. *World Journal of Gastroenterology* 10: 1802–1805.

Simon, O., A. Jadamus, and W. Vahjen. 2001. Probiotic feed additives – effectiveness and expected modes of action. *Journal of Animal Feed Sciences* 10: 51–57.

Simon, O., W. Vahjen, and D. Taras. 2004. Interaction of nutrition with intestinal microbial communities. In *Interfacing immunity, gut health and performance*, ed. L.A. Tucker and J.A. Taylor-Pickard, 33–46. Nottingham: Nottingham University Press.

Smirnov, A., E. Tako, P. Ferket, and Z. Uni. 2006. Mucin gene expression and mucin content in the chicken intestinal goblet cells are affected by in ovo feeding of carbohydrates. *Poultry Science* 85: 669–673.

Smith, H.W. 1965. The development of the flora of the alimentary tract in young animals. *J Path Bact* 90: 495–513.

Smits, H.M., A. Veldman, M.W.A. Verstegen, and A.C. Beynen. 1997. Dietary carboxymethyl cellulose with high instead of low viscosity reduces macro nutrient digestion in broiler chickens. *Journal of Nutrition* 127: 483–487.

Steer, T., H. Carpenter, K. Tuohy, and G.R. Gibson. 2000. Perspectives on the role of the human gut microbiota and its modulation by pro and prebiotics. *Nutrition Research Reviews* 13: 229–254.

Sterzo, H., J.B. Paiva, A.L. Mesquita, O.C. Freitas Neto, and A. Berchieri Jr. 2007. Organic acids and/or compound with defined microorganisms to control *Salmonella enterica* serovar Enteritidis experimental infection in chickens. *British Journal of Poultry Science* 9: 69–73.

Suskovic, J., B. Kos, J. Goreta, and S. Matosic. 2001. Role of lactic acid bacteria and bifidobacteria in synbiotic effect. *Food Technology and Biotechnology* 39: 227–235.

Taherpour, K., H. Moravej, M. Shivazad, M. Adibmoradi, and B. Yakhchali. 2009. Effects of dietary probiotic, prebiotic and butyric acid glycerides on performance and serum composition in broiler chickens. *African Journal of Biotechnology* 8: 2329–2334.

Timmerman, H.M., A. Veldman, E. van den Elsen, F.M. Rombouts, and A.C. Beynen. 2006. Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. *Poultry Science* 85: 1383–1388.

Torok, V.A., K. Ophel-Keller, R.J. Hughes, R. Forder, M. Ali, and R. Macalpine. 2007. Environment and age: Impact on poultry gut microflora. In *Proceedings of 19th Australian poultry science symposium*, 149–152. Sydney: Poultry Research Foundation.

Tuohy, K.M., G.C.M. Rouzaud, W.M. Bruck, and G.R. Gibson. 2005. Modulation of the human gut microflora towards improved health using prebiotics-assessment of efficacy. *Current Pharmaceutical Design* 11: 75–90.

Turner, J.L., S.S. Dritz, and J.E. Minton. 2001. Alternatives to conventional antimicrobials in swine diets. *Professional Animal Scientist* 17: 217–226.

Uni, Z., S. Ganot, and D. Sklan. 1998. Posthatch development of mucosal function in the broiler small intestine. *Poultry Science* 77: 75–82.

Uni, Z., Y. Noy, and D. Sklan. 1999. Posthatch development of small intestine function in the poult. *Poultry Science* 78: 215–222.

USDA. 2007a. *Hatchery production 2006 summary*. Washington, DC: National Agricultural Statistics Service, USDA.

USDA. 2007b. *Progress report on Salmonella testing of raw meat and poultry products, 1998–2006*. Washington, DC: Food Safety and Inspection Service, USDA.

Van Immerseel, F., R. Ducatelle, M. DeVos, N. Boon, et al. 2010. Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease. *Journal of Medical Microbiology* 59: 141–143.

Virden, W.S., and M.T. Kidd. 2009. Physiological stress in broilers: Ramifications on nutrient digestibility and responses. *Journal of Applied Poultry Research* 18: 338–347.

Voetsch, A.C., T.J. Van Gilder, F.J. Angulo, M.M. Farley, et al. 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clinical Infectious Diseases* 38(Suppl 3): 127–134.

Vugia, D., A. Cronquist, J. Hadler, M. Tobin-D'Angelo, et al. 2007. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – 10 states, 2006. *Morbidity and Mortality Weekly Report* 56: 336–339.

Vugia, D., A. Cronquist, M. Carrter, M. Tobin-D'Angelo, et al. 2009. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – 10 states, 2008. *Morbidity and Mortality Weekly Report* 58: 333–337.

Wadstrom, T., K. Andersson, M. Sydow, L. Axelsson, S. Lindgren, and B. Gullmar. 1987. Surface properties of *Lactobacilli* isolated from the small intestine of pigs. *Journal of Applied Bacteriology* 62: 513–520.

Wang, J., C. Jenkins, R.I. Webb, and J.A. Fuerst. 2002. Isolation of *Gemmata*-like and *Isosphaera*-like planctomycete bacteria from soil and freshwater. *Applied and Environmental Microbiology* 68: 417–422.

Williams, B.A., M.W.A. Verstegen, and S. Tamminga. 2001. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. *Nutrition Research Reviews* 14: 207–227.

Willis, W.L., and L. Reid. 2008. Investigating the effects of dietary probiotic feeding regimens on broiler chicken production and *Campylobacter jejuni* presence. *Poultry Science* 87: 606–611.

Woodward, C.L., Y.M. Kwon, L.F. Kubena, J.A. Byrd, R.W. Moore, D.J. Nisbet, and S.C. Ricke. 2005. Reduction of *Salmonella enterica* serovar Enteritidis colonization and invasion by an alfalfa diet during molt in Leghorn hens. *Poultry Science* 84: 185–193.

Yamauchi, K., and J. Snel. 2000. Transmission electron microscopic demonstration of phagocytosis and intracellular processing of segmented filamentous bacteria by intestinal epithelial cells of the chick ileum. *Infection and Immunity* 68: 6496–6504.

Yurong, Y., S. Ruiping, Z. Shimin, and J. Yibao. 2005. Effect of probiotics on intestinal mucosal immunity and ultrastructure of cecal tonsils of chickens. *Archives of Animal Nutrition* 59: 237–246.

Zeweil, H.S., S.G. Genedy, and M. Bassiouni. 2006. Effect of probiotic and medicinal plant supplements on the production and egg quality of laying Japanese quail hens. In *Proceeding of the 12th European poultry conference., ZWANS*, 1–6. Verona. http://lba.zwans.com/fullpapers/10224.pdf.

Zhu, X.Y., T. Zhong, Y. Pandya, and R.D. Joerger. 2002. 16s rRNA-based analysis of microbiota from the caecum of broiler chickens. *Applied and Environmental Microbiology* 68: 124–137.

Chapter 8
Current Status of Practical Applications: Probiotics in Dairy Cattle

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Abstract The gastrointestinal microbial population of dairy cattle is dense and diverse and can be utilized to reduce pathogenic bacterial populations as well as improve animal productivity and environmental effects. Because of the nature of the dairy industry, probiotic products have been widely used to enhance milk production and the feed efficiency. The individual efficacy of probiotics in dairy cattle is due to specific microbial ecological factors within the gut of the food animal and its native microflora that alter the competitive pressures of the gut. This chapter explores the ecology behind the efficacy of probiotic products against food-borne pathogens that inhabit food animals.

8.1   Introduction

In the United States, the dairy industry includes approximately nine million cattle that produce, on average, 19,000 pounds of milk a year (USDA-ERS 2009). During the past four decades the number of dairy farms has decreased, whereas the average dairy herd size has increased (USDA-ERS 2009). This concentration of cattle on fewer but larger farms has occurred to meet the demands of economies of scale. This focus on improving the efficiency of milk production has led to the development of dietary strategies to improve this critical factor in farm profitability (Losinger

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"Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, or exclusion of others that may be suitable."

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and Heinrichs 1996). To achieve this goal, dairy cattle are fed a variety of rations from very high grain diets, to total mixed rations (TMRs), to even solely grass-based grazing systems. Many of the diets fed to dairy cattle in recent years have included the use of probiotic feedstuffs, or direct-fed microbials (DFMs).

The gastrointestinal (GI) tract of cattle is a fully mature ecosystem comprised of more than 600 known species of bacteria as well as protozoa and fungi (Hungate 1966). This mixed, diverse microbial consortium occupies all environmental niches and utilizes nearly all available nutrients (Stewart and Bryant 1988). The symbiotic relationship between the host animal and its resident gastrointestinal microbial eco-system is critical to animal health and production efficiency (Jayne-Williams and Fuller 1971; Savelkoul and Tijhaar 2007). The ability to utilize cellulose has allowed ruminant animals to occupy environmental niches free of competition, but this has come at a cost of relatively low feed efficiencies (Stewart and Bryant 1988). Utilization of the native or an artificially introduced microbial population to improve some aspect of animal production has been termed a "probiotic," or a competitive enhancement approach (Fuller 1989). Generally speaking, these approaches offer a natural "green" method to improve production, efficiency, and safety of dairy production.

8.2   Which DFMs Are Used in Dairy Cattle?

Direct-fed microbials comprise a general category of dietary products that can be included in animal rations to enhance performance and/or reduce the number of pathogenic bacteria (Collins and Gibson 1999; Fuller 1989). DFMs are microorganisms that have "generally recognized as safe" (GRAS) status, are labeled in accordance with the Association of American Feed Control Officials (AAFCO), and in the United States are regulated by the Food and Drug Administration (FDA). Included in this definition of DFMs are products used in animals and humans, such as probiotics, prebiotics, competitive exclusion cultures, and enzyme preparations (Schrezenmeir and De Vrese 2001). Enzyme preparations are not discussed in this chapter as we, instead, focus on the category of "probiotic" approaches. Although prebiotics and competitive exclusion cultures are utilized in some phases of animal production, their use to date in dairy cattle has been quite limited because of the presence of the rumen and its microbial population (Brashears et al. 2003b; Kaufhold et al. 2000; Moxley et al. 2003). There has, however, been simultaneous use of pre-biotics and a probiotic – known as "synbiotics" – which has been used experimentally in cattle (Yasuda et al. 2007).

Most of the DFMs used in dairy cattle fall into the category of probiotics. The dairy cattle industry has used various DFMs for years primarily to increase the growth rate, milk production, and/or production efficiency (Dawson 1990; Lehloenya et al. 2008). Recent years have seen the development of probiotic preparations to address other concerns related to dairy production and cattle health. Probiotic preparations

that are used in dairy cattle are typically individual species or mixtures of lactic acid bacteria (LABs), yeasts, or their end-products and are not species-specific (not limited to use in cattle) or even necessarily originally isolated from animals (Wiemann 2003). Probiotics often fall into three categories (1) live cultures of yeast or bacteria; (2) heat-treated (or otherwise inactivated) cultures of yeast or bacteria; (3) fermentation end-products from incubations of yeast or bacteria. All of these probiotic categories have been used in various stages of lactation or growth in dairy cattle. Regulations in this field have allowed a wide variety of claims to be made about the improvements in growth efficiency and other potential benefits, and consistency of results in the field has not always been demonstrated (Barroga et al. 2007; LeJeune et al. 2006).

Yeast and fungal products are some of the most widely utilized DFM products in the dairy industry (Callaway and Martin 2006; Dawson 1990; Dawson 1992). Often yeast/fungal products are fed as live or dead products, and they may or may not include fermentation end-products. The most common members of this type used in dairy cattle include cultures of *Saccharomyces cerevisiae*, *Aspergillus oryzae,* and *Aspergillus niger* (Isik et al. 2004; Yoon and Stern 1996). Bacterial DFM products are typically comprised of LABs and are not necessarily originally isolated from animals (Wiemann 2003). However, the most commonly used probiotic bacterial strains in dairy cattle are *Bifidobacterium*, *Propionibacterium*, *Enterococcus* (*Streptococcus*), and *Lactobacillus*. The bacterial probiotics are primarily targeted for improving milk production and feed conversion efficiency (Gomes and Malcata 1999; Midilli et al. 2008; Nocek and Kautz 2006), although in some cases these bacterial species have been coupled with feeding indigestible (at least by the animal) sugars to yield a symbiotic effect (Yasuda et al. 2007).

8.3 Why Are DFMs Used in Cattle?

Cattle are inherently inefficient in converting feed to milk or meat because of the symbiotic relationship between the cow and her resident ruminal microbial ecosystem (Hungate 1966). Feed efficiency in dairy cattle typically ranges from 3 to nearly 1, depending on the stage of lactation, parity, dietary composition and digestibility, body composition, genetics, animal health, environmental conditions, and other management factors (Linn and Salfer 2006). Feed efficiency, in turn, has a significant impact on the profitability of a dairy farm (Linn and Salfer 2006), one that can mean the difference between a successful operation and one that fails. The use of DFMs in dairy cattle rations has improved feed efficiency in some studies and conditions (Nocek and Kautz 2006; Oetzel et al. 2007; Raeth-Knight et al. 2007) and as a result has produced a positive impact on dairy profitability (Desnoyers et al. 2009).

Another reason for giving probiotic products to dairy cattle involves food and environmental safety. Each year more than 27% of the U.S. population is sickened by food-borne pathogenic bacteria (Scharff 2010; USDA-ERS 2001). The indirect

and direct cost each year of the five most common food-borne pathogenic bacteria in the United States totals more than US\$40 billion (Scharff 2010). Food-borne pathogenic bacteria can be harbored asymptomatically in the gut of dairy cattle or on their hides (Arthur et al. 2007; Doyle and Erickson 2006; Porter et al. 1997; Reid et al. 2002). Pathogenic bacteria such as enterohemorrhagic *Escherichia coli* (including *E. coli* O157:H7), *Salmonella*, *Campylobacter*, and *Listeria* have all been isolated from cattle (Callaway et al. 2006; Harvey et al. 2004; Oliver et al. 2005).

Although it is a food safety concern, *Salmonella* can also cause severe disease in cattle and is a problem both from food safety and animal health perspectives (Coburn et al. 2007; USDA/APHIS 2003a, 2003b). Waste streams emanating from dairy farms are being viewed increasingly in some regions of the United States as a threat to the environment and to public health (Ibekwe et al. 2002). Thus asymptomatic carriage of pathogenic bacteria represents a threat to the integrity and the efficiency and profitability of milk production. Consequently, strategies to reduce animal health/food safety pathogens in various phases of dairy cattle production have been developed, including the development of targeted probiotics.

8.4   How Does DFM Feeding Benefit Dairy Cattle?

The results of probiotic studies in dairy cattle over the years have unfortunately been characterized by inconsistency, primarily due to a lack of understanding of the microbial ecology of the GI tract and those of the probiotic organisms utilized. Some probiotic microorganisms chosen for use in dairy cattle were isolated from other animal species or other environments and were thus not ecologically suited for life in the gut of the target species. Additionally, variations between studies can be attributed to antagonistic interactions among some probiotic species obtained "over the counter." Furthermore, mature animals contain a stable, relatively individualistic intestinal microbial population with which the probiotic must come into equilibrium; when probiotics are applied to calves, results tend to be more consistent (Chiquette et al. 2007; Isik et al. 2004; Krehbiel et al. 2003; Nader-MacÃas et al. 2008). All of these factors have contributed to difficulties in reproducing effects of some probiotics in animals beyond the neonatal stage.

In recent years, advances in molecular methodologies have allowed more precise characterization of each probiotic, improved monitoring of the specific changes caused by individual probiotic cultures, and provided a better understanding of the "normal" gut flora and degree of individualization of the intestinal microbial ecosystem. These advances can lead to the development of highly tailored probiotic products for use in production situations (i.e., lactation and the dry period). However, because these advances are still in their infancy, they and their implications are not discussed in depth.

8.4.1   Effects of DFMs on Dairy Cattle Production and Performance

Direct-fed microbials are included in dairy rations primarily to improve milk production efficiency (Isik et al. 2004; Jouany 2006; Oetzel et al. 2007). Regulations in the area of probiotics have allowed a wide variety of claims to be made about the improvements in growth efficiency and other potential benefits, and consistency of results in the field has not always been demonstrated (Desnoyers et al. 2009; Krehbiel et al. 2003). However, the most common claim of probiotics in relation to dairy cattle is an improvement in feed efficiency and/or milk production. Many studies have demonstrated that probiotic products can enhance production efficiency and thus improve dairy farm profitability (Desnoyers et al. 2009); yet this can vary widely based on the product type (i.e., fungal versus bacterial; live culture versus fermentation extract), organism selected, diet that the cattle are fed, and the stage of lactation (Windschitl 1992).

The most widely used probiotic bacterial strains in the cattle industry are *Propionibacterium* and *Lactobacillus* (Gomes and Malcata 1999), although in recent years the use of typical ruminal bacteria has been examined to improve dietary efficiency of cattle. The addition of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* to the diet of mid-lactation cattle had no impact on the dry matter intake (DMI), feed efficiency, or milk production (Raeth-Knight et al. 2007). A *Lactobacillus*-based probiotic fed singly and in combination with a *S. cerevisiae* (yeast) culture showed no change in milk production or efficiency in early-lactation dairy cows (Boga and Gorgulu 2007). Other researchers have found that feeding a *Propionibacterium* decreased plasma glucose and insulin concentrations in cattle (Aleman et al. 2007). In other studies examining the inclusion of propionibacteria-based DFMs, it was observed that daily milk yield and fat-corrected milk yield were increased across a 30-week lacation interval, but that no change in reproductive parameters was noted (Stein et al. 2006). When early-lactation cows were treated with the same *Propionibacterium* strain, total ruminal volatile fatty acid (VFA) concentrations were increased, but the milk yield remained unchanged. However, when coupled with a decrease in DMI, the energetic efficiency was increased by DFM feeding (Weiss et al. 2008). In a pair of studies examining the effect of an *Enterococcus faecium* DFM on cows immediately before freshening found that postpartum blood glucose levels were increased, along with milk yield and DMI (Nocek and Kautz 2006; Nocek et al. 2003). A mixture of yeast and *Propionibacterium* feeding increased plasma glucose and increased milk production by mid-lactation but not during early lactation (Lehloenya et al. 2008).

Bacteria comprise an estimated 40% of the biomass of the bovine ruminal microbial population, and these prokaryotes are relatively well understood (Hungate 1966). To date, however, there has been little use for ruminal bacteria reintroduced into the ruminant as a DFM. Inclusion of the ruminal bacterium *Ruminococcus flavefaciens* increased digestibility of hay but was required to be fed daily for this bacterium to remain in the rumen (Chiquette et al. 2007). Feeding a *Prevotella bryantii* probiotic

to early-lactation cattle resulted in an increase in milk fat concentration and ruminal fermentation products (Chiquette et al. 2008). Other studies using the lactate-utilizing ruminal bacterium *Megasphaera elsdenii* found that addition of this bacterium could reduce ruminal lactate accumulation in ruminal fermentations, at least in vitro (Kung and Hession 1995). Other researchers have hypothesized that specific ruminal bacteria could be used to target bacterial species that produce lactate and waste dietary energy, but the effects of the addition of this bacterium on ruminal fermentation has not been determined in vivo (Wells et al. 1997).

The most common fungal DFM products fed to dairy cattle include those made from the yeast *Sacharomyces* and the fungus *Aspergillus*. In general, some of the fungal DFM preparations are live cultures, whereas others are not alive (fermentation extracts). It appears that, after feeding, the nonliving cultures act more as a prebiotic than a probiotic per se, although they are not marketed as such. Many of the live cultures must be continuously fed to maintain detectable ruminal populations of these products (Kung et al. 1997). Similarly, the fermentation extracts must be fed daily to maintain their benefits. In an outstanding meta-analysis of yeast DFM feeding studies, Desnoyers et al. found that yeast supplementation increased rumen pH and VFA concentrations and decreased the ruminal lactic acid concentrations; yet it had no effect on the acetate/propionate ratio (Desnoyers et al. 2009). DMI, milk yield, and fat-corrected milk also were also increased by yeast supplementation (Desnoyers et al. 2009).

Researchers found that feeding live cultures and fermentation extracts of *Aspergillus oryzae* increased ruminal pH and the VFA concentration, although no synbiotic effect was detected (Oellermann et al. 1990; Wiedmeirer et al. 1987). Other studies found that addition of *Aspergillus oryzae* cultures had more impact regarding increased ruminal pH and VFA production in animals on low-forage diets than in animals on high-forage diets (Gomez-Alarcon et al. 1990). In other studies, milk yields and production efficiency were improved in early-lactation cows fed a high grain diet supplemented with an *Aspergillus oryzae* culture, but the effects were less pronounced in mid-lactation cows(Gomez-Alarcon et al. 1991). In still other studies, supplementation with *Aspergillus oryzae* had no impact on DMI, milk yield, or diet digestibility (Sievert and Shaver 1993). When fungal and yeast cultures were compared directly, it was found that ruminal pH, ammonia nitrogen concentration, and total VFA concentration were similar (Yoon and Stern 1996). Interestingly, the percentages of ruminal isoacids were lower for the cows that were fed a mixture of yeast and fungal cultures than when fed either culture alone. Overall, the yeast culture increased digestibility of OM and CP, but fungal cultures stimulated the cellulolytic bacterial counts (Yoon and Stern 1996).

In in vitro studies, the addition of *Saccharomyces cerevisiae* to fermentations decreased lactate accumulation and methane production (Lila et al. 2004). The inclusion of the yeast *S. cerevisiae* culture in dairy cow rations with relatively high concentrate levels caused an increase in DMI and increased milk yield (Williams et al. 1991). This study also demonstrated a decrease in lactate accumulation and a decrease in the acetate/propionate ratio (Williams et al. 1991). Feeding another live yeast product (*Saccharomyces*) to mid-lactation cattle improved milk production significantly (Kung et al. 1997).

Collectively, the evidence supports the fact that DFMs (bacterial or fungal) can improve milk production and production efficiency in dairy cows (Desnoyers et al. 2009). The results have not always been consistent in magnitude, however; the reasons behind this variability are still unknown but may be elucidated with the advent of molecular population estimates of the rumen and intestinal tract of cattle. Many of the benefits of DFM feeding appear to be greatest in animals undergoing stress or transitions (e.g., parturition). DFMs appear to make their greatest contribution to improving production in situations where animals are exposed to hot weather (Yu et al. 1997), low-quality diets, or other stresses.

8.4.2 Health Benefits of DFM

Subacute ruminal acidosis (SARA) is a condition associated with the consumption of large amounts of readily fermentable grain by cattle (Enemark 2008). A major end-product of the rapid fermentation of starch or glucose, often by streptococci or lactobacilli, is lactic acid (Owens et al. 1998). This powerful acid lowers the pH of the ruminal fluid and keratinizes the ruminal epithelium (Slyter 1976). As the ruminal pH decreases, lactic acid bacteria proliferate at the expense of other members of the microbial ecosystem, leading to "typhooning" of ruminal conditions and leading to acidosis in the animal (Russell and Hino 1984). hen cattle are mildly acidotic, they are subject to cyclic feeding (and associated production disruption) as well as to peritonitis, liver abscesses, and laminitis (Nagaraja and Titgemeyer 2007). Because dairy cattle are often maintained on high grain rations for long periods of time, chronic SARA is often found in these animals (Enemark 2008). Milk-fat depression was once a critical negative effect of SARA; but given the general shift away from a milkfat-differential-based economic model, it is not as important a factor in farm profitability as it once was. Reduced DMI, cyclic feeding, and milk production decreases caused by SARA do affect production efficiency and profit. Many of the probiotics used in the dairy industry produce an increase in ruminal pH to combat the typhooning effect of SARA before it affects milk production or animal health (Chiquette 2009; Desnoyers et al. 2009; Jouany 2006).

In addition to the reduction in SARA achieved by some probiotic preparations, DFM can also be used in dairy cattle as direct disease preventatives (Nader-MacÃas et al. 2008). The addition of bovine vaginal lactic acid bacteria (primarily) as a probiotic preparation inhibited the growth of metritis-causing organisms in dairy cattle (Otero et al. 2006; Otero and Nader-MacÃas 2006). This is largely due to anti-staphylococcal activity of a probiotic H_2O_2-producing *Lactobacillus* isolated from cattle vaginas (Otero and Nader-MacÃas 2006). Other research on probiotic preparations found that the bacteria living on the surface of a healthy udder could inhibit the in vitro growth of mastitis-causing organisms, including *Aracanobacterium pyogenes* (Al-Qumber and Tagg 2006). Another *Lactobacillus* culture has been shown to reduce mastitis (Crispie et al. 2008), which is possibly linked to stimulation of the immune response through up-regulation of interleukin-1 (IL-1) and IL-8 in the mammary gland (Beecher et al. 2009).

Johne's disease is a disease in dairy cattle caused by *Mycobacterium avium* ssp. paratuberculosis; it manifests in ways similar to the human Crohn's disease. Other researchers have found that cultures of the bacterium *Dietzia* spp. can reduce myco-bacterial populations in adult dairy cattle (Click and van Kampen 2009). In another study, calves were fed a mixture of *Saccharomyces cerevisiae*, *Lactobacillus acido-philus*, *Bifidobacterium bifidum*, *Streptococcus* thermophilus, and *Aspergillus niger*, which improved weight gain and reduced the incidence of scours (Isik et al. 2004). Although these are not yet market-ready products, these results emphasize the fact that probiotic approaches have a wide application in preventing animal diseases and ensuring animal productivity.

8.4.3   *Food Safety Benefits of DFM*

Because of the U.S. Food Safety Inspection Service's declaration of *E. coli* O157:H7 an adulterant in ground beef, there has been intensified interest in probiotic research aimed at reducing *E. coli* O157:H7 in both beef and dairy cattle (Krehbiel et al. 2003; Oliver et al. 2005; Oliver et al. 2008; Stefan 1997). Early researchers in this topic found that a variety of commercial probiotics provided neither benefit nor detriment in regard to *E. coli* O157:H7 populations in cattle (Keen and Elder 2000). One DFM based on LABs reduced fecal shedding of *E. coli* O157:H7 in sheep (Lema et al. 2001). In later results, an *L. acidophilus* culture reduced *E. coli* O157:H7 shedding by more than 50% in finishing cattle (Brashears et al. 2003a, 2003b). Additional research indicated that this commercial DFM reduced fecal shedding of *E. coli* O157:H7 in cattle from 46% of animals to 13% (Ransom et al. 2003). Other research demonstrated that this DFM reduced *E. coli* O157:H7 populations on the hides of cattle by up to 75%; furthermore, the highest DFM dosage reduced *Salmonella* shedding in the feces by 50% (Stephens et al. 2007b; Younts-Dahl et al. 2004). Studies have also shown a continuous positive impact from the feeding of *Lactobacillus* and a greater impact when *Propionibacterium* was included with the *Lactobacillus* (Elam et al. 2003; Stephens et al. 2007a). As a result, a LAB probiotic product (Bovamine) is currently used widely in feedlots across the United States and Canada because the enhanced growth performance economically balances the cost of its inclusion in cattle rations, thus making a food safety enhancement eco-nomically viable, at least in beef cattle. In another study, a different DFM that also included *L. acidophilus* significantly reduced fecal shedding of *E. coli* O157:H7; fecal shedding of *Salmonella* (which can also be an animal health threat) was not reduced in cattle, but there were fewer new *Salmonella* infections (Tabe et al. 2008). To date, the effects of these LAB DFMs on dairy production have not been reported, so their utility to the dairy industry cannot be estimated.

8.5   DFM Modes of Action

The synergistic relationship between the host animal and its GI microbial ecosystem is critical to the health and well-being of the animal and to efficient production (Jayne-Williams and Fuller 1971). Recent studies have demonstrated that certain members of the microbial population of the gut (in humans, at least) can have an effect on obesity and are linked to conditions such as autism (DiBaise et al. 2008; Finegold 2008; Ley et al. 2006). Thus, it is not surprising that altering the composition of the GI microbiome of dairy cattle can alter the composition or amount of milk produced as well as the animal's health and well-being (Lock and Bauman 2004). However, the mechanisms behind this alteration can be quite different than the traditional model of implanting a live dominant microbial population in the GI tract.

Some DFMs that are fed to dairy cattle are comprised in whole or in part of compounds that fall into the category of "prebiotics," which are indigestible by the animal but available to the microbial population (Crittenden 1999). The use of DFMs containing prebiotics can provide nutrients to sustain specific microbial populations in the rumen and intestinal tract of dairy cows, potentially helping to select for a more efficient microbial population (from the animal's perspective). Ruminal methane production can account for a loss of up to 12% of the carbon and energy fed to cows (Johnson and Johnson 1995). Some dairy DFMs have been noted to have an "ionophore-like" (e.g., reduced methane production, increased pH, reduced ammonia excretion) effect on ruminal fermentation (Bergen and Bates 1984). Much of the beneficial effect of DFMs in the dairy industry has been linked to a reduction in the production of methane in the rumen (Boadi et al. 2004). Some of the dairy yeast-based DFMs have been noted to have an "ionophore-like" effect, which has been attributed, at least in part, to the presence of dicarboxylic acids (e.g., fumarate and malate), which act in some cases as a prebiotic (Martin et al. 1999; Nisbet and Martin 1990). The inclusion of these acids stimulates the growth and utilization of lactic acid by predominant ruminal bacterial species, which can greatly affect the ruminal pH, methane production, and total VFA profile (Jouany 2006; Martin et al. 1999; Tejido et al. 2005).

Other dairy DFM products contain bacteriocins/colicins (or bacterocinogenic bacterial/yeast species), which are natural antimicrobial proteins produced by bacteria or yeasts. These potent antimicrobials can alter the microbial population in the GI tract (Ross et al. 2010). The inclusion of bacteriocins or colicins to improve animal health and food safety has been suggested, and recent innovations from molecular biology have increased the economic feasibility of this form of DFM (Federic and Sokol 1973; Schamberger and Diez-Gonzalez 2002; Vosough Ahmadi et al. 2007). Other ways in which DFMs may benefit dairy cattle and dairy producers is through the production (or inclusion in fermentation products) of B vitamins that are absorbed by the host animal (Branner and Roth-Maier 2006). Also, the alteration (or introduction of a stable) intestinal microbial population can stimulate the immune system (Koenen et al. 2004; Schierack et al. 2007), which can reduce colonization by pathogens and subsequent illnesses. Some probiotic products have been shown to affect immune

parameters directly and increase CD8 production and IgG and IgM concentrations in the serum and gut (Duncker et al. 2006; Walsh et al. 2008; Zhang et al. 2008) and IL-1 and IL-8 expression in dairy cattle (Beecher et al. 2009).

8.6   Conclusions

The diversity of the microbial population of the ruminant intestinal tract is a natural resource that we can now harness to improve animal health and performance. One of the best mechanisms we have for harnessing this resource is the use of probiotics and prebiotics (DFMs). Addition of ruminal and intestinal microbial populations from healthy animals or stimulation of an existing normal intestinal flora may establish a normal, diverse, more efficient microbial population. DFMs appear, at least in some cases, to improve the efficiency of milk production in dairy cows. The energetic status of the animal and its production demands necessarily play an important role in the degree and significance of the impact of DFM feeding on milk production. DFMs improve animal performance and health through a variety of hypothesized mechanisms that are still not fully understood. However, by enhancing our knowledge of how the microbial population in and on the animal affects its growth, we can further enhance growth efficiency, productivity, food safety, and animal health.

References

Aleman, M.M., D.R. Stein, D.T. Allen, E. Perry, K.V. Lehloenya, T.G. Rehberger, K.J. Mertz, D.A. Jones, and L.J. Spicer. 2007. Effects of feeding two levels of propionibacteria to dairy cows on plasma hormones and metabolites. *Journal of Dairy Research* 74: 146–153.

Al-Qumber, M., and J.R. Tagg. 2006. Commensal bacilli inhibitory to mastitis pathogens isolated from the udder microbiota of healthy cows. *Journal of Applied Microbiology* 101: 1152–1160.

Arthur, T.M., J.M. Bosilevac, X. Nou, S.D. Shackleford, T.L. Wheeler, and M. Koohmaraie. 2007. Comparison of the molecular genotypes of *Escherichia coli* O157:H7 from the hides of beef cattle in different regions of North America. *Journal of Food Protection* 70: 1622–1626.

Barroga, A.J., R.C.D. Salas, E.A. Martin, W.G. Besa, and C.A.D.C. Santos. 2007. Efficacy of probiotics, enzymes and dried porcine solubles in swine. *Philippine Agricultural Scientist* 90: 71–74.

Beecher, C., M. Daly, D.P. Berry, K. Klostermann, J. Flynn, W. Meaney, C. Hill, T.V. McCarthy, R.P. Ross, and L. Giblin. 2009. Administration of a live culture of *Lactococcus lactis* DPC 3147 into the bovine mammary gland stimulates the local host immune response, particularly IL-1 and IL-8 gene expression. *Journal of Dairy Research* 76: 340–348.

Bergen, W.G., and D.B. Bates. 1984. Ionophores: Their effect on production efficiency and mode of action. *Journal of Animal Science* 58: 1465–1483.

Boadi, D., C. Benchaar, J. Chiquette, and D. Masse. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. *Canadian Journal of Animal Science* 84: 319–335.

Boga, M., and M. Gorgulu. 2007. Effects of probiotics based on *Lactobacillus* sp. and *Lactobacillus* sp. plus yeast (*Sacchoromyces cerevisiae*) on milk yield and milk composition of dairy cows. *Cuban Journal of Agricultural Science* 41: 305–308.

Branner, G.R., and D.A. Roth-Maier. 2006. Influence of pre-, pro-, and synbiotics on the intestinal availability of different B-vitamins. *Archives of Animal Nutrition* 60: 191–204.

Brashears, M.M., M.L. Galyean, G.H. Loneragan, J.E. Mann, and K. Killinger-Mann. 2003a. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *Journal of Food Protection* 66: 748–754.

Brashears, M.M., D. Jaroni, and J. Trimble. 2003b. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. *Journal of Food Protection* 66: 355–363.

Callaway, T.R., and S.A. Martin. 2006. *Use of fungi and organic acids in production animal diets. Feedstuffs direct-fed microbial, enzyme and forage additive compendium*, 8th ed, 25–33. Minneton: Miller Publishing, Inc.

Callaway, T.R., T.S. Edrington, A.D. Brabban, J.E. Keen, R.C. Anderson, M.L. Rossman, M.J. Engler, K.J. Genovese, B.L. Gwartney, J.O. Reagan, T.L. Poole, R.B. Harvey, E.M. Kutter, and D.J. Nisbet. 2006. Fecal prevalence of *Escherichia coli* O157, *Salmonella*, *Listeria*, and bacteriophage infecting *E. coli* O157:H7 in feedlot cattle in the southern plains region of the United States. *Foodborne Pathogens and Disease* 3: 234–244.

Chiquette, J. 2009. Evaluation of the protective effect of probiotics fed to dairy cows during a subacute ruminal acidosis challenge. *Animal Feed Science and Technology* 153: 278–291.

Chiquette, J., G. Talbot, F. Markwell, N. Nili, and R.J. Forster. 2007. Repeated ruminal dosing of *Ruminococcus flavefaciens* NJ along with a probiotic mixture in forage or concentrate-fed dairy cows: Effect on ruminai fermentation, cellulolytic populations and in sacco digestibility. *Canadian Journal of Animal Science* 87: 237–249.

Chiquette, J., M.J. Allison, and M.A. Rasmussen. 2008. *Prevotella bryantii* 25A used as a probiotic in early-lactation dairy cows: Effect on ruminal fermentation characteristics, milk production, and milk composition. *Journal of Dairy Science* 91: 3536–3543.

Click, R.E., and C.L. van Kampen. 2009. Short communication: Progression of Johne's disease curtailed by a probiotic. *Journal of Dairy Science* 92: 4846–4851.

Coburn, B., G.A. Grassl, and B.B. Finlay. 2007. *Salmonella*, the host and disease: A brief review. *Immunology and Cell Biology* 85: 112–118.

Collins, D.M., and G.R. Gibson. 1999. Probiotics, prebiotics, and synbiotics: Approaches for modulating the microbial ecology of the gut. *The American Journal of Clinical Nutrition* 69: 1052S–1057S.

Crispió, F., M. Alonso-Gómez, C. O'Loughlin, K. Klostermann, J. Flynn, S. Arkins, W. Meaney, R. Paul Ross, and C. Hill. 2008. Intramammary infusion of a live culture for treatment of bovine mastitis: Effect of live lactococci on the mammary immune response. *Journal of Dairy Research* 75: 374–384.

Crittenden, R.G. 1999. Prebiotics. In *Probiotics: A critical review*, ed. G.W. Tannock, 141–156. Wymondham: UK, Horizon Scientific Press.

Dawson, K.A. 1990. Designing the yeast culture of tomorrow – Mode of action of yeast culture for ruminants and non-ruminnts. *Biotechnology in the Feed Industry* 5: 59–75.

Dawson, K.A. 1992. Current and future role of Yeast culture in animal production: A review of research over last six years. *Animal Science* 8: 1–23.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Ponter, and D. Sauvant. 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. *Journal of Dairy Science* 92: 1620–1632.

DiBaise, J.K., H. Zhang, M.D. Crowell, R. Krajmalnik-Brown, G.A. Decker, and B.E. Rittmann. 2008. Gut microbiota and its possible relationship with obesity. *Mayo Clinic Proceedings* 83: 460–469.

Doyle, M.P., and M.C. Erickson. 2006. Reducing the carriage of food-borne pathogens in livestock and poultry. *Poultry Science* 85: 960–973.

Duncker, S.C., A. Lorentz, B. Schroeder, G. Breves, and S.C. Bischoff. 2006. Effect of orally administered probiotic *E. coli* strain Nissle 1917 on intestinal mucosal immune cells of healthy young pigs. *Veterinary Immunology and Immunopathology* 111: 239–250.

Elam, N.A., J.F. Gleghorn, J.D. Rivera, M.L. Galyean, P.J. Defoor, M.M. Brashears, and S.M. Younts-Dahl. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal character-istics, and *Escherichia coli* strain O157 shedding of finishing beef steers. *Journal of Animal Science* 81: 2686–2698.

Enemark, J.M.D. 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *The Veterinary Journal* 176: 32–43.

Federic, F., and A. Sokol. 1973. Effect of peroral application of a colicinogenic strain of *Escherichia coli* on the incidence of different categories of plasmids in *Escherichia coli* isolated from weaned piglets fed on flavomycin. *Folia Microbiologica* 18: 65.

Finegold, S.M. 2008. Therapy and epidemiology of autism-clostridial spores as key elements. *Medical Hypotheses* 70: 508–511.

Fuller, R. 1989. Probiotics in man and animals. *The Journal of Applied Bacteriology* 66: 365–378.

Gomes, A.M.P., and F.X. Malcata. 1999. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: bio-logical, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends in Food Science and Technology* 10: 139–157.

Gomez-Alarcon, R.A., C. Dudas, and J.T. Huber. 1990. Influence of cultures of *Aspergillus oryzae* on rumen and total tract digestibility of dietary components. *Journal of Dairy Science* 73: 703–710.

Gomez-Alarcon, R.A., J.T. Huber, G.E. Higginbotham, F. Wiersma, D. Ammon, and B. Taylor. 1991. Influence of feeding *Aspergillus oryzae* fermentation extract on the milk yields, eating patterns, and body temperatures of lactating cows. *Journal of Dairy Science* 72: 1733–1740.

Harvey, R.B., R.E. Droleskey, C.L. Sheffield, T.S. Edrington, T.R. Callaway, R.C. Anderson, D.L.J. Drinnon, R.L. Ziprin, H.M. Scott, and D.J. Nisbet. 2004. Campylobacter prevalence in lactating dairy cows in the United States. *Journal of Food Protection* 67: 1476–1479.

Hungate, R.E. 1966. *The rumen and its microbes*. New York: Academic.

Ibekwe, A.M., P.M. Watt, C.M. Grieve, V.K. Sharma, and S.R. Lyons. 2002. Multiplex fluorogenic real-time PCR for detection and quantification of *Escherichia coli* O157:H7 in dairy wastewa-ter wetlands. *Applied and Environmental Microbiology* 68: 4853–4862.

Isik, M., F. Ekimler, N. Ozen, and M.Z. Firat. 2004. Effects of using probiotics on the growth performance and health of dairy calves. *Turkish Journal of Veterinary and Animal Sciences* 28: 63–69.

Jayne-Williams, D.J., and R. Fuller. 1971. The influence of the intestinal microflora on nutrition. In *Physiology and biochemistry of the domestic food*, ed. D.J. Bell and B.M. Freeman, 74–92. London: Academic Press.

Johnson, K.A., and D.E. Johnson. 1995. Methane emissions from cattle. *Journal of Animal Science* 73: 2483–2494.

Jouany, J.P. 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. *Animal Reproduction Science* 96: 250–264.

Kaufhold, J., H.M. Hammon, and J.W. Blum. 2000. Fructo-oligosaccharide supplementation: Effects on metabolic, endocrine and hematological traits in veal calves. *Journal of Veterinary Medicine A: Physiology, Pathology, Clinical Medicine* 47: 17–29.

Keen, J., and R. Elder. 2000. Commercial probiotics are not effective for short-term control of entero-hemorrhagic *Escherichia coli* O157 infection in beef cattle. Kyoto, Japan, 4th International Symposium Works. Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* Infect., 92 (Abstr.).

Koenen, M.E., J. Kramer, R. Van Der Hulst, L. Heres, S.H.M. Jeurissen, and W.J.A. Boersma. 2004. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. *British Poultry Science* 45: 355–366.

Krehbiel, C.R., S.R. Rust, G. Zhang, and S.E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *Journal of Animal Science* 81: E120–132.

Kung, L., and A.O. Hession. 1995. Preventing in vitro lactate accumulation in ruminal fermenta-tions by inoculation with *Megasphaera elsdenii*. *Journal of Animal Science* 73: 250–256.

Kung, L., E.M. Kreck, R.S. Tung, A.O. Hession, A.C. Sheperd, M.A. Cohen, H.E. Swain, and J.A.Z. Leedle. 1997. Effects of a live yeast culture and enzymes on in vitro ruminal fermentation and milk production of dairy cows. *Journal of Dairy Science* 80: 2045–2051.

Lehloenya, K.V., D.R. Stein, D.T. Allen, G.E. Selk, D.A. Jones, M.M. Aleman, T.G. Rehberger, K.J. Mertz, and L.J. Spicer. 2008. Effects of feeding yeast and propionibacteria to dairy cows on milk yield and components, and reproduction. *Journal of Animal Physiology and Animal Nutrition* 92: 190–202.

LeJeune, J.T., M.D. Kauffman, M.D. Amstutz, and L.A. Ward. 2006. Limited effects of a commercial direct-fed microbial on weaning pig performance and gastrointestinal microbiology. *Journal of Swine Health and Production* 14: 247–252.

Lema, M., L. Williams, and D.R. Rao. 2001. Reduction of fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. *Small Ruminant Research* 39: 31–39.

Ley, R.E., P.J. Turnbaugh, S. Klein, and J.I. Gordon. 2006. Human gut microbes associated with obesity. *Nature* 444: 1022–1023.

Lila, Z.A., N. Mohammed, T. Yasui, Y. Kurokawa, S. Kanda, and H. Itabashi. 2004. Effects of a twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation in vitro. *Journal of Animal Science* 82: 1847–1854.

Linn, J.G., and J. Salfer. 2006. *Feed efficiency*. Minneapolis: University of Minnesota. Extension Service.

Lock, A.L., and D.E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* 39: 1197–1206.

Losinger, W.C., and A.J. Heinrichs. 1996. Dairy operation management practices and herd milk production. *Journal of Dairy Science* 79: 506–514.

Martin, S.A., M.N. Streeter, D.J. Nisbet, G.M. Hill, and S.E. Williams. 1999. Effects of DL-malate on ruminal metabolism and performance of cattle fed a high-concentrate diet. *Journal of Animal Science* 77: 1008–1015.

Midilli, M., M. Alp, N. Kocabagli, O.H. Muglali, N. Turan, H. Yilmaz, and S. Cakir. 2008. Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. *South African Journal of Animal Sciences* 38: 21–27.

Moxley, R.A., D. Smith, T.J. Klopfenstein, G. Erickson, J. Folmer, C. Macken, S. Hinkley, A. Potter, and B. Finlay. 2003. Vaccination and feeding a competitive exclusion product as intervention strategies to reduce the prevalence of *Escherichia coli* O157:H7 in feedlot cattle. In *Edinburgh, UK, Proceedings 5th International Symposium on Shiga Toxin-Producing Escherichia coli Infections*, 23 (Abstr.).

Nader-MacÃas, M.E.F., M.C. Otero, M.C. Espeche, and N.C. Maldonado. 2008. Advances in the design of probiotic products for the prevention of major diseases in dairy cattle. *Journal of Industrial Microbiology and Biotechnology* 35: 1387–1395.

Nagaraja, T.G., and E.C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. *Journal of Dairy Science* 90(Suppl. 1): E17–38.

Nisbet, D.J., and S.A. Martin. 1990. Effect of dicarboxylic acids and *Aspergillus oryzae* fermentation extract on lactate uptake by the ruminal bacterium *Selenomonas ruminantium*. *Applied and Environmental Microbiology* 56: 3515–3518.

Nocek, J.E., and W.P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. *Journal of Dairy Science* 89: 260–266.

Nocek, J.E., W.P. Kautz, J.A.Z. Leedle, and E. Block. 2003. Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. *Journal of Dairy Science* 86: 331–335.

Oellermann, S.O., M.J. Arambel, B.A. Kent, and J.L. Walters. 1990. Effect of graded amounts of *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility in cattle. *Journal of Dairy Science* 73: 2413–2416.

Oetzel, G.R., K.M. Emery, W.P. Kautz, and J.E. Nocek. 2007. Direct-fed microbial supplementation and health and performance of pre- and postpartum dairy cattle: A field trial. *Journal of Dairy Science* 90: 2058–2068.

Oliver, S.P., B.M. Jayarao, and R.A. Almeida. 2005. Food-borne pathogens in milk and the dairy farm environment: Food safety and public health implications. *Foodborne Pathogens and Disease* 2: 115–129.

Oliver, S.P., D.A. Patel, T.R. Callaway, and M.E. Torrence. 2008. ASAS centennial paper: Developments and future outlook for preharvest food safety. *Journal of Animal Science* 87: 419–437.

Otero, M.C., and M.E. Nader-MacÃas. 2006. Inhibition of *Staphylococcus aureus* by H_2O_2-producing *Lactobacillus gasseri* isolated from the vaginal tract of cattle. *Animal Reproduction Science* 96: 35–46.

Otero, M.C., L. Morelli, and M.E. Nader-Macias. 2006. Probiotic properties of vaginal lactic acid bacteria to prevent metritis in cattle. *Letters in Applied Microbiology* 43: 91–97.

Owens, F.N., D.S. Secrist, W.J. Hill, and D.R. Gill. 1998. Acidosis in cattle: A review. *Journal of Animal Science* 76: 275–286.

Porter, J., K. Mobbs, C.A. Hart, J.R. Saunders, R.W. Pickup, and C. Edwards. 1997. Detection, distribution, and probable fate of *Escherichia coli* O157 from asymptomatic cattle on a dairy farm. *Journal of Applied Microbiology* 83: 297–306.

Raeth-Knight, M.L., J.G. Linn, and H.G. Jung. 2007. Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of holstein dairy cows. *Journal of Dairy Science* 90: 1802–1809.

Ransom, J.R., K.E. Belk, J.N. Sofos, J.A. Scanga, M.L. Rossman, G.C. Smith, and J.D. Tatum. 2003. *Investigation of on-farm management practices as pre-harvest beef microbiological interventions*. Centennial: National Cattlemen's Beef Association Research Fact Sheet.

Reid, C.A., A. Small, S.M. Avery, and S. Buncic. 2002. Presence of food-borne pathogens on cattle hides. *Food Control* 13: 411–415.

Ross, R.P., S. Mills, C. Hill, G.F. Fitzgerald, and C. Stanton. 2010. Specific metabolite production by gut microbiota as a basis for probiotic function. *International Dairy Journal* 20: 269–276.

Russell, J.B., and T. Hino. 1984. Regulation of lactate production in *Streptococcus bovis*: A spiraling effect that contributes to rumen acidosis. *Journal of Dairy Science* 68: 1712–1721.

Savelkoul, H., and E. Tijhaar. 2007. Animal health and immunomodulation of the natural defense system. *Dierg. immunomod. natuurl. weer.* 132: 764–766.

Schamberger, G.P., and F. Diez-Gonzalez. 2002. Selection of recently isolated colicinogenic *Escherichia coli* strains inhibitory to *Escherichia coli* O157:H7. *Journal of Food Protection* 65: 1381–1387.

Scharff, R.L. 2010. *Health-related costs from food-borne illness in the United States*. Washington: Georgetown University.

Schierack, P., L.H. Wieler, D. Taras, V. Herwig, B. Tachu, A. Hlinak, M.F.G. Schmidt, and L. Scharek. 2007. *Bacillus cereus* var. *toyoi* enhanced systemic immune response in piglets. *Veterinary Immunology and Immunopathology* 118: 1–11.

Schrezenmeir, J., and M. De Vrese. 2001. Probiotics, prebiotics, and synbiotics-approaching a definition. *The American Journal of Clinical Nutrition* 73(Suppl.): 354s–361s.

Sievert, S.J., and R.D. Shaver. 1993. Carbohydrate and *Aspergillus oryzae* effects on intake, digestion, and milk production by dairy cows. *Journal of Dairy Science* 76: 245–254.

Slyter, L.L. 1976. Influence of acidosis on rumen function. *Journal of Animal Science* 43: 910–929.

Stefan, G. 1997. Food safety issues affecting the dairy beef industry. *Journal of Dairy Science* 80: 3458–3462.

Stein, D.R., D.T. Allen, E.B. Perry, J.C. Bruner, K.W. Gates, T.G. Rehberger, K. Mertz, D. Jones, and L.J. Spicer. 2006. Effects of feeding propionibacteria to dairy cows on milk yield, milk components, and reproduction. *Journal of Dairy Science* 89: 111–125.

Stephens, T.P., G.H. Loneragan, L.M. Chichester, and M.M. Brashears. 2007a. Prevalence and enumeration of *Escherichia coli* O157 in steers receiving various strains of Lactobacillus-based direct-fed microbials. *Journal of Food Protection* 70: 1252–1255.

Stephens, T.P., G.H. Loneragan, E. Karunasena, and M.M. Brashears. 2007b. Reduction of *Escherichia coli* O157 and *Salmonella* in feces and on hides of feedlot cattle using various doses of a direct-fed microbial. *Journal of Food Protection* 70: 2386–2391.

Stewart, C.S., and M.P. Bryant. 1988. *The rumen microbial ecosystem*. London: Elsevier Science Publishers, Ltd.

Tabe, E.S., J. Oloya, D.K. Doetkott, M.L. Bauer, P.S. Gibbs, and M.L. Khaitsa. 2008. Comparative effect of direct-fed microbials on fecal shedding of *Escherichia coli* O157:H7 and *Salmonella* in naturally infected feedlot cattle. *Journal of Food Protection* 71: 539–544.

Tejido, M.L., M.J. Ranilla, R. García-Martinez, and M.D. Carro. 2005. In vitro microbial growth and rumen fermentation of different substrates as affected by the addition of disodium malate. *Journal of Animal Science* 81: 31–38.

USDA/APHIS. 2003a. *Salmonella* and *Campylobacter* on U. S. dairy operations. USDA/APHIS-VS Centers for Epidemiology and Animal Health.

USDA/APHIS. 2003b. *Salmonella* and *Listeria* in bulk tank milk on U.S. dairies. USDA/APHIS-VS Centers for Epidemiology and Animal Health.

USDA-ERS. 2001. *ERS estimates food-borne disease costs at $6.9 billion per year*. Economic Research Service-United States Department of Agriculture.

USDA-ERS. 2009. *Farm milk production*. Economic Research Service, USDA.

Vosough Ahmadi, B., K. Frankena, J. Turner, A.G.J. Velthuis, H. Hogeveen, and R.B.M. Huirne. 2007. Effectiveness of simulated interventions in reducing the estimated prevalence of *E. coli* O157:H7 in lactating cows in dairy herds. *Veterinary Research* 38: 755–771.

Walsh, M.C., G.E. Gardiner, O.M. Hart, P.G. Lawlor, M. Daly, B. Lynch, B.T. Richert, S. Radcliffe, L. Giblin, C. Hill, G.F. Fitzgerald, C. Stanton, and P. Ross. 2008. Predominance of a bacteriocin-producing *Lactobacillus salivarius* component of a five-strain probiotic in the porcine ileum and effects on host immune phenotype. *FEMS Microbiology Ecology* 64: 317–327.

Weiss, W.P., D.J. Wyatt, and T.R. McKelvey. 2008. Effect of feeding propionibacteria on milk production by early lactation dairy cows. *Journal of Dairy Science* 91: 646–652.

Wells, J.E., D.O. Krause, T.R. Callaway, and J.B. Russell. 1997. A bacteriocin-mediated antagonism by ruminal lactobacilli against *Streptococcus bovis*. *FEMS Microbiology Ecology* 22: 237–243.

Wiedmeirer, R.D., M.J. Arambel, and J.L. Walters. 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. *Journal of Dairy Science* 70: 2063–2068.

Wiemann, M. 2003. How do probiotic feed additives work? *International Poultry Production* 11: 7–9.

Williams, P.E.V., C.A.G. Tait, G.M. Innes, and C.J. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. *Journal of Animal Science* 69: 3016–3026.

Windschitl, P.M. 1992. Effects of probiotic supplementation of hull-less barley- and corn-based diets on bacterial fermentation in continuous culture of ruminal contents. *Canadian Journal of Animal Science* 72: 265–272.

Yasuda, K., S. Hashikawa, H. Sakamoto, Y. Tomita, S. Shibata, and T. Fukata. 2007. A new synbiotic consisting of *Lactobacillus casei* subsp. *casei* and dextran improves milk production in Holstein dairy cows. *The Journal of Veterinary Medical Science* 69: 205–208.

Yoon, I.K., and M.D. Stern. 1996. Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* cultures on ruminal fermentation in dairy cows. *Journal of Dairy Science* 79: 411–417.

Younts-Dahl, S.M., M.L. Galyean, G.H. Loneragan, N.A. Elam, and M.M. Brashears. 2004. Dietary supplementation with Lactobacillus- and Propionibacterium-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. *Journal of Food Protection* 67: 889–893.

Yu, P., J.T. Huber, C.B. Theurer, K.H. Chen, L.G. Nussio, and Z. Wu. 1997. Effect of steam-flaked or steam-rolled corn with or without *Aspergillus oryzae* in the diet on performance of dairy cows fed during hot weather. *Journal of Dairy Science* 80: 3293–3297.

Zhang, W., M.S.P. Azevedo, A.M. Gonzalez, L.J. Saif, T. Van Nguyen, K. Wen, A.E. Yousef, and L. Yuan. 2008. Influence of probiotic Lactobacilli colonization on neonatal B cell responses in a gnotobiotic pig model of human rotavirus infection and disease. *Veterinary Immunology and Immunopathology* 122: 175–181.

Chapter 9
Current and Future Status of Practical Applications: Beef Cattle

Blake K. Wilson and Clinton R. Krehbiel

Abstract The feeding of direct-fed microbials (DFMs) has received much consideration from the beef cattle industry. This is due in part to a current public perception that there is a need for sufficient disease prevention while simultaneously reducing the utilization of antimicrobials in beef production. Probiotics have been long used in the beef industry as a method to improve cattle health and productivity. In this chapter, evidence regarding the use of some of the DFMs used in beef cattle are explored, and the benefits and challenges of inclusion of these feedstuffs in the diet are addressed. Changes in rumen function and the microbial ecosystem and effects on carcass merit are also addressed.

9.1 Introduction

The feeding of direct-fed microbials (DFMs) has received much consideration from the beef cattle industry. This is due in part to a current public perception that there is a need for sufficient disease prevention while simultaneously reducing the utilization of antimicrobials in beef production (Krehbiel et al. 2003; Raeth-Knight et al. 2007). At the same time, the reduction in antimicrobial use must be achieved without losing the current advantages of production efficiency. Trying to find ways to accomplish this has been an area of concentrated research in recent years. DFMs have been a well-received alternative in beef cattle diets because they contain a source of live, naturally occurring microorganisms (Yoon and Stern 1995; AAFCO 1999; FDA 2003).

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The original concept of feeding a DFM to cattle was based on the presumption of potential benefits on intestinal effects, which included the establishment of more desirable microflora and prevention of the establishment of pathogenic organisms (Krehbiel et al. 2003). Data suggest that feeding a DFM to cattle decreases the fecal shedding of *Escherichia coli* O157:H7 (Brashears et al. 2003; Elam et al. 2003; Younts-Dahl et al. 2005; Tabe et al. 2008; Callaway et al. 2009). Other beneficial responses observed when providing bacterial DFMs to cattle include increases in average daily gains and improved feed efficiency in feedlot cattle; improved health, increased immunity, and increased performance in young calves; decreased potential for ruminal acidosis; increased propionate concentration in the rumen; and altered rumen microflora populations (Krehbiel et al. 2003; Guillen 2009).

9.2 History of Direct-Fed Microbials and Application in Beef Cattle

Probiotics, or DFMs, have a long and intriguing history. Probiotics have been defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller 1989). Some consider the terms probiotics and DFMs interchangeable. Probiotics, however, is a generic and all-encompassing term used for microbial cultures, extracts, and enzyme preparations; and it is commonly used when the product is for human consumption (Elam et al. 2003). The preferred term when used in reference to products fed to livestock is DFMs. The U.S. Food and Drug Administration (FDA) and the Association of American Feed Control Officials (AAFCO) have required feed manufacturers to use the term "direct-fed microbial" instead of probiotic in animal feeds (Miles and Bootwalla 1991; AAFCO 1999; FDA 2003). Furthermore, the FDA has gone on to define DFMs as "a source of live, naturally occurring microorganisms" (Yoon and Stern 1995; Krehbiel et al. 2003).

Metchnikoff, who is considered the father of probiotics, first proposed that it was desirable to consume live lactobacilli capable of living inside the gastrointestinal (GI) tract (Gilliland 1989; Yoon and Stern 1995). Metchnikoff was searching for the always intriguing fountain of youth and studied the life-spans of people in other parts of the world. He theorized that the longevity of Bulgarian people was due to their consumption of a fermented milk product that contained lactobacilli (Gilliland 1989; Yoon and Stern 1995; Krehbiel et al. 2003). In 1908, Metchnikoff published a book, *The Prolongation of Life*, that outlined his findings and theories. This book led to several studies on *Lactobacillus* species during the 1920s (Stern and Storrs 1975). The early popularity of *Lactobacillus acidophilus* therapy reached its peak during the 1930s (Stern and Storrs 1975). Following World War I and II, the widespread use and effectiveness of antibiotics that often destroyed all intestinal bacteria led to an increase of "antibiotic diarrhea," which led to renewed interest in *L. acidophilus* therapy for intestinal microflora repair and restoration (Krehbiel et al. 2003).

In recent years, there have been increasing societal concerns over the use of antibiotics and other growth stimulants in the livestock industry. This situation is further complicated by the increased emphasis placed on the industry to reduce diseases and pathogens while simultaneously improving production efficiency. The combination of these two concerns has led to an increased interest in the effects of DFMs on animal health and performance (Krehbiel et al. 2003). The original concept of feeding a DFM to livestock was based on the presumption of potential beneficial intestinal effects, which included establishing a more desirable microflora and preventing the establishment of pathogenic organisms (Krehbiel et al. 2003). Some additional responses to bacterial DFMs in cattle include increases in average daily gains and improved feed efficiency in feedlot cattle; improved health, increased immunity, and increased performance in young calves; decreased potential for ruminal acidosis; increased propionate concentrations in the rumen; and altered rumen microflora populations (Krehbiel et al. 2003; Guillen 2009).

Currently, there are at least 42 individual species of microorganisms that are approved for use in DFMs by the FDA and AAFCO (Alliance Animal Health 2009). The two DFM species most commonly fed to ruminants are *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* (Raeth-Knight et al. 2007). The feeding of these two organisms together is thought to be advantageous because of the individual characteristics of each organism. *Lactobacillus acidophilus* is a lactate-producing bacterium, and *Propionibacterium freudenreichii* is a lactate-utilizing bacterium and produces propionate resulting from fermentation (Raeth-Knight et al. 2007).

9.3 Use of Direct-Fed Microbials in Diets for Growing and Finishing Beef Cattle

Society's concerns over the continued use of antibiotics in production agriculture and the increased interest in disease and pathogen prevention in the food supply have led to an increased interest in use of DFMs in growing and finishing cattle (Elam et al. 2003). Other, more economical reasons for the increase in usage of DFM products in growing and finishing cattle include improved performance, improved health responses in sick cattle, and significantly reduced mortality in heavier cattle (Krehbiel et al. 2003; McDonald et al. 2005). Cattle weighing ≥318 kg had significantly reduced death loss when receiving a DFM (McDonald et al. 2005).

Although studies in newly received cattle or stocker cattle are limited, the results of these studies suggest that the use of a DFM can improve the health and performance of stressed or newly received cattle (Krehbiel et al. 2003). Feeding a single dose of a DFM to steer calves prior to the initiation of grazing spring wheat in the pasture improved performance (Phillips et al. 2005). Upon arrival to the feedlot, newly received feeder cattle typically have ruminal bacterial populations that are adept at digesting forage but not starch. As cattle are "stepped up" from high-forage diets to high-grain diets, the microbial population in the rumen shifts toward species

that can utilize starch. Once cattle have acclimated to a high-starch diet, there is most likely a benefit to shifting toward the more efficient microbial species and away from those that are less efficient and/or most likely to produce lactic acid. DFMs can potentially be involved in this process.

As cattle are placed on feed and are stepped up toward high levels of the finishing diet, feeding lactate-producing bacteria could help prevent acidosis because the presence of these bacteria allows the ruminal microorganisms to adapt to the controlled presence of lactic acid in the rumen (Yoon and Stern 1995). Once the population has shifted to one that is capable of fermenting high-starch diets, and the animal has learned to regulate intake based on chemostatic rather than physical fill, there might be further benefit in using DFMs to shift the population toward the more efficient, consistent, and safer fermentation end-products such as propionate. The feeding of lactate-utilizing bacteria such as *Propionibacterium* might result in increased propionic acid production and diminished lactic acid production. This change would increase the recovery of energy from the diet and provide a potential basis for increased average daily gain and improved feed efficiency in cattle fed DFMs.

To give an indication of the extent of DFM use in feedlots, McDonald et al. (2005) evaluated the VetLife Benchmark Performance Program survey. Data from this VetLife survey in 2004 confirmed the widespread use of DFMs in feedlots (McDonald et al. 2005). The survey regarding DFM usage in feedlots received responses from 267 feedlots and records on 10,900,504 cattle. Of the 267 feedlots surveyed in this study, 118 were using some form of a DFM product (McDonald et al. 2005). This amounted to more than 44% of feedlots in the study that were using a DFM product at the time of the survey. Many estimate even more widespread uses of DFM products today.

9.4   Direct-Fed Microbials and Rumen Function

In the rumen, microorganisms convert feed into volatile fatty acids, which enter the portal circulation and are used by the gut, liver, and peripheral tissues to provide energy to the animal. Microbial populations also provide protein and energy via the lower gut, as a portion of them are constantly washed out of the rumen and digested by mammalian enzymes in the abomasum and small intestine. DFMs might alter the species composition of the ruminant bacterial population, resulting in changes in fermentation that could be beneficial to the host animal. Because the fermentation characteristics of the bacterial species differ, it could alter rumen function. Some bacterial species have lower maintenance energy requirements than others or produce energy sources that can be used more efficiently by the animal. If the population is shifted toward an increase in these "favorable" bacterial species, the result could be improved energy utilization by the host.

In addition to energy efficiency, rumen health could be improved by feeding a DFM (Krehbiel et al. 2003). An inherent risk of feeding high-energy diets to cattle is

the conversion of energy-dense feed to acids in the rumen. As starch is converted by microbes to acid and the acid accumulates, the ruminal pH is lowered. If the acid accumulates faster than the rumen can absorb it, rumen health is compromised and rumen function is impaired, resulting in bloat or ruminal acidosis. Acute ruminal acidosis or chronic acidosis due to the ingestion of excessive amounts of readily fermentable carbohydrate is a prominent production problem in beef cattle fed high-concentrate diets (Owens et al. 1998). Ruminal acidosis has been characterized by a decrease in ruminal pH (≤5.6 for subacute ruminal acidosis and ≤5.2 for acute ruminal acidosis) and high ruminal concentrations of total volatile fatty acids in the case of subacute acidosis or lactic acid during acute acidosis. If too much acid enters the bloodstream, the health of the animal can be compromised even to the point of death. These acid overload situations generally result when the animal overeats or when the rumen bacterial population is unprepared to deal with high-energy feed. Although the mode of action of DFMs in the rumen is not completely understood, the presence of lactate-producing bacteria is thought to help the ruminal microflora adapt to the presence of lactic acid (Ghorbani et al. 2002), whereas the presence of lactate-utilizing bacteria is thought to prevent accumulation of lactate (Nisbet and Martin 1994; Kung and Hession 1995). Therefore, continual inoculation with certain bacterial DFMs might help the ruminal environment adapt acidosis (Elam 2003).

Few studies have characterized the effects of bacterial DFMs on ruminal and total tract digestion of nutrients. Beauchemin et al. (2003) reported that in sacco ruminal digestion of corn, barley, and alfalfa hay were decreased when *Enterococcus faecium* was fed. In contrast, *Propionibacterium* or *Propionibacterium* and *E. faecium* did not affect the in sacco disappearance of dry matter (Ghorbani et al. 2002). In the study by Beauchemin et al. (2003), supplementation with bacterial DFMs had no effect on the site or extent of starch digestion. However, supplementing diets with *E. faecium* tended to decrease the total tract digestibility of organic matter and intestinal digestion of neutral detergent fiber. Beauchemin et al. (2003) suggested that the lower digestion of fiber by steers supplemented with *E. faecium* might have been associated with the observed lower ruminal pH. In continuous culture, the digestibility of dry matter, organic matter, starch, neutral detergent fiber, acid detergent fiber, and nitrogen was not affected by bacterial DFM supplementation (Yang et al. 2004). In vivo, however, feeding *E. faecium* tended to decrease the flow of microbial nitrogen from the rumen and increased the flow of feed nitrogen (Beauchemin et al. 2003). Decreased flow of microbial nitrogen from the rumen resulted from the numerical decrease in efficiency of microbial protein synthesis. Although results are inconsistent, the available literature generally suggests that bacterial DFMs have minimal effects on ruminal digestibility of nutrients.

Although digestibility data are limited, several studies have characterized the effects of DFMs on ruminal pH and proportions of volatile fatty acids. Results from studies using beef cattle supplemented with *Lactobacillus* species have shown a lower area under the ruminal pH curve (Huffman et al. 1992; Nocek et al. 2002), suggesting reduced risk of subacute ruminal acidosis when DFMs were fed. For example, ruminally fistulated steers were fed a 50% concentrate diet for 12 days and subsequently dosed (day 13) with a 100% concentrate diet via a ruminal cannula to

induce subacute acidosis (Huffman et al. 1992). Supplementing daily with 5×10^8 cfu of *Lactobacillus acidophilus* decreased the amount of time that ruminal pH was <6.0 compared with control steers. Similarly, ruminal pH in steers receiving *Lactobacillus* was <6.0 for fewer hours during a 24-h period than control steers when cannulated steers were induced with ruminal acidosis by intraruminally dosing a 50:50 blend of fine ground corn and dry-rolled wheat (1.6% of body weight) (Lodge et al. 1996). Nocek et al. (2002) provided ruminally cannulated dairy cows fed a 70% concentrate diet with a mixture of *E. faecium*, *L. acidophilus*, and *Saccharomyces cerevisiae* (yeast) at 10^5, 10^6, or 10^7 cfu/ml of ruminal fluid daily and measured ruminal fermentation characteristics. Compared with the control group, cows inoculated with 10^5 cfu/ml of ruminal fluid had the highest mean daily ruminal fluid pH and the fewest mean daily hours of ruminal pH <5.5. Based on this work, Nocek et al. (2002) suggested that DFMs that produce lactate sustain a tonic level of lactic acid in the rumen, which could potentially stimulate lactic acid-utilizing bacteria.

Other groups have reported no effect of bacterial DFMs on ruminal pH. For example, Ghorbani et al. (2002) reported no effect of 10 g/steer·day^{-1} of a carrier that contained *Propionibacterium* P15 or *Propionibacterium* P15 and *E. faecium* EF212 (1×10^9 cfu/g) on ruminal pH (average pH 5.71) in steers adapted to an 87% steam-rolled barley diet. Similarly, Beauchemin et al. (2003) evaluated the effects of *E. faecium* (6×10^9 cfu/day), a lactate-producing bacterium, alone or with yeast (*S. cerevisiae* 6×10^9 cfu/day) and reported no effect of treatment on the proportion of time or area pH <5.8 or <5.5. Beauchemin et al. (2003) noted that the incidence of subclinical aciodosis was more severe in their experiment than in previously published experiments. In their experiment, the ruminal pH of steers was <5.5 for ≥39% of the day (Beauchemin et al. 2003). In a companion study with similar treatments using continuous culture, the mean fermenter pH, as well as the lowest and highest pH, were not affected by bacterial DFMs (Yang et al. 2004). Beauchemin et al. (2003) indicated that the lack of an effect of bacterial DFMs on ruminal pH suggests little benefit of providing DFMs that either produce or utilize lactic acid when the rumen is adapted to a high-grain diet. However, the authors noted that in feeding situations in which lactic acid might accumulate in the rumen providing bacterial DFMs might prove beneficial.

Van Koevering et al. (1994) reported that ruminal concentrations of D-lactate and total lactate were decreased for steers supplemented with *L. acidophilus* BT 1389, regardless of the dietary concentrate level (92% or 55% concentrate). In contrast, steers consuming an 87% steam-rolled barley diet supplemented with *Propionibacterium* P15 or *Propionibacterium* and *E. faecium* EF212 had ruminal concentrations of L-lactate and total volatile fatty acids similar to those of control steers (Ghorbani et al. 2002). Similarly, Beauchemin et al. (2003) reported no effect of *E. faecium* or *E. faecium* and *S. cerevisiae* (yeast) on the total volatile fatty acid concentration, and the lactate concentration was below detection limits in steers fed an 87% steam-rolled barley diet. These results suggest that the effect of bacterial DFM supplementation on decreasing ruminal acidosis depends on the amount of lactic acid accumulation during ruminal fermentation. Continued research with different bacterial DFM species and combinations using acidosis challenge models might be beneficial (Beauchemin et al. 2006).

In in vitro and in vivo studies, *Megasphaera elsdenii* inoculation has modified ruminal fermentation and prevented the accumulation of lactic acid during the transition from low- to high-concentrate diets (Greening et al. 1991; Kung and Hession 1995). The pH of cultures treated with *M. elsdenii* (8.7×10^6 cfu/ml of culture fluid) was decreased to <5.5 at 4 h and remained at approximately 5.3 for 24 h, whereas the control pH was decreased to 4.8 (Kung and Hession 1995). Lactate concentration peaked at more than 40 mM in controls after 8 h and remained fairly constant thereafter, whereas after *M. elsdenii* treatment it was <5 mM throughout incubation. The total volatile fatty acid concentration of cultures treated with *M. elsdenii* was more than twice that of control (131.4 vs. 63.3 mM). Most differences in volatile fatty acid concentration between treatments resulted from increased butyrate, valerate, and branched-chain fatty acids (Kung and Hession 1995). *Propionibacterium* or the combination of *Propionibacterium* and *E. faecium* did not affect ruminal fluid concentrations of propionate, isobutyrate, or isovalerate, or the acetate/propionate ratio (Ghorbani et al. 2002). However, the acetate concentration was greater for steers receiving *Propionibacterium* and *E. faecium* than for steers receiving *Propionibacterium* alone or no bacterial DFM. In addition, Ghorbani et al. (2002) reported that steers fed *Propionibacterium* alone had greater concentrations of ruminal butyrate. Other researchers (Slyter et al. 1992; Kung and Hession 1995) have reported accumulation of butyrate when *M. elsdenii* is grown in pure culture, and Lodge et al. (1996) reported a lower acetate plus butyrate/propionate ratio for control steers compared with steers receiving a combined *Lactobacillus* and yeast DFM. Lodge et al. (1996) suggested that the production of propionate might be decreased during an acidosis challenge in steers supplemented with DFMs.

In contrast with the aforementioned experiments, Beauchemin et al. (2003) reported that supplementing an 87% concentrate diet with *E. faecium* increased the proportion of propionate and decreased the proportion of butyrate in ruminal fluid compared with control steers. As indicated by the authors, results in their study were consistent with the expectation that supplementing *E. faecium*, a lactate utilizer, would increase propionate. Similarly, Kim et al. (2000) studied the effect of increasing dosage levels (none, 10^7, 10^8, 10^9, and 10^{10} cfu) of *Propionibacterium acidipropionici* on ruminal fermentation in steers fed a high-concentrate diet. When supplemented with *P. acidipropionici*, all dosage levels had numerically lower concentrations of acetate and greater concentrations of propionate, and therefore the acetate/propionate ratio decreased at all dosages except 10^8 cfu. It appears that *P. acidipropionici* altered ruminal metabolism toward less acetate and more propionate production. In addition, the ruminal butyrate concentration decreased as the dose of *P. acidipropionici* increased; and when *P. acidipropionici* was removed, the butyrate concentration returned to near pretest levels. Although reasons for discrepancies among experiments are difficult to explain, these data suggest that the energy favorable propionate concentration might be increased in ruminants fed high-grain diets when supplemented with a lactate-utilizing DFM.

Ghorbani et al. (2002) reported that feeding *Propionibacterium* increased protozoa (especially *Entodinium*) and decreased amylolytic bacteria in the rumen of feedlot steers. Protozoal numbers were significantly increased in *Propionibacterium* steers over controls and *Propionibacterium* and *E. faecium* steers. Amylolytic

bacterial numbers were significantly decreased in steers during *Propionibacterium* supplementation compared with counts from controls and *Propionibacterium* and *E. faecium*-supplemented steers (Ghorbani et al. 2002). Although the mechanism by which bacterial DFMs stimulate protozoa remain unclear, Ghorbani et al. (2002) indicated that the decrease in amylolytic species was likely the result of the increase in protozoal numbers because protozoa are predators of ruminal bacteria. Similar to the results of Ghorbani et al. (2002), Van Koevering et al. (1994) reported that including cultures of *L. acidophilus* BT 1389 in the diet prolonged retention of protozoa in steers fed a 92% concentrate diet. In contrast, supplementing the diet with *E. faecium* tended to decrease protozoal numbers but had no effect on lactate-utilizing bacteria, amylolytic bacteria, or total bacterial numbers (Beauchemin et al. 2003). In continuous culture, counts of total bacteria in the fermenter fluid tended to be greater when the control or *Propionibacterium* P15 was included compared with *E. faecium* or *E. faecium* and yeast (Yang et al. 2004). However, lactate-utilizing bacterial numbers were greater for controls or *E. faecium* than for *Propionibacterium* or *E. faecium* and yeast. As suggested by the authors, one would anticipate that the number of lactate-utilizing bacteria would be greater when *Propionibacterium* was fed, in contrast with the data.

In summary, the potential for DFMs to decrease the risk or severity of ruminal acidosis in cattle fed high-concentrate diets is still in question. As suggested by Elam et al. (2003), the inconsistency of the data does not provide unequivocal evidence that DFMs are efficacious for decreasing episodes of ruminal and/or metabolic acidosis. In addition, more work is needed to enhance our understanding of factors involved with variation in ruminal volatile fatty acids and lactate concentrations when DFMs are fed. Responses most likely depend on the species of bacterial DFMs fed and may be different if a yeast product is included.

9.5   Direct-Fed Microbials and Beef Cattle Health

There is substantial research suggesting that DFMs have favorable effects on the health of beef cattle. Krehbiel et al. (2003) reviewed the available literature on DFMs and observed that eight publications reported favorable effects of DFMs given at processing or in the feed at arrival on either health or performance of newly received calves. Favorable health effects of DFMs may be situation-dependent, as four of the studies reviewed reported no positive health effects of feeding a DFM. This could be a result of the health risk of the cattle used, experimental design, product delivery, or the DFMs tested (Brown and Nagaraja 2009). Gill et al. (1987) speculated that extremely sick or extremely healthy calves might be less likely to show a health benefit from DFMs.

The published data indicate that DFMs demonstrate a supportive role in the health of feedlot cattle, offering improved recovery and performance in most situations, but most likely not as therapeutic treatment. Both research (Krehbiel et al. 2003) and population data (McDonald et al. 2005) have shown performance

benefits of DFMs, and these advantages are likely the primary reasons that cattle feeders choose to use DFM products. However, potential ancillary benefits of DFMs include higher or more consistent feed consumption, fewer digestive disorders or digestion-related deaths, and improved health or response to treatment.

McDonald et al. (2005) reported that positive trends were observed for health-related variables. Improved health manifests as reduced death loss or increased performance for animals at high risk of morbidity. McDonald et al. (2005) showed performance benefits of DFMs in lots of cattle with substantial health risk. In pens of cattle that had $20 or less in processing and treatment costs, average daily gain advantages were 4.5% and 3.1% in steers and heifers, respectively, with feed/gain advantages of 1%–2%. For cattle with more than $20 per head of processing and treatment charges, the advantages of DFMs were even greater. These data suggest that cattle with modest health challenges (less than $20 per animal of processing and treatment costs) respond favorably to DFMs, but cattle with greater health challenges (more than $20 per animal) have a higher rate of response to DFMs. Therefore, DFM products may allow cattle to extract more energy from feed, encourage greater dry matter intake, or contribute to more rapid recovery from an immune challenge.

Death loss among 320- to 365-kg cattle has been shown to be lower for pens of cattle fed DFMs than for those without them (0.85% and 1.01%, respectively) (McDonald et al. 2005). Although DFMs decreased death loss among heavy cattle, the data did not indicate the same effect in lighter cattle. Death loss was not significantly lower for DFM-fed cattle that were <320 kg compared to cattle of similar weight that were not fed a DFM. This difference may be explained by the frequency and most common causes of death in the different weight groups. In the United States, cattle <320 kg placed on feed in 2003 had an average death loss of 2.21%, whereas cattle that weighed 320–400 kg had 0.95% death loss (McDonald et al. 2005). In the lighter groups most of the deaths were likely due to respiratory causes with a few digestive deaths, whereas most of the deaths in the heavier cattle were likely due to digestive disorders such as acidosis and bloat (McDonald et al. 2005). DFMs would be expected to have the most direct effect on digestive deaths, which would be most common in heavy cattle.

9.6 Direct-Fed Microbials and Beef Cattle Performance

Direct fed microbials can affect feedlot cattle performance positively. Supplementing feedlot diets on a daily basis with lactate-producing and/or lactate-utilizing bacteria has been shown to improve average daily gains and feed efficiency of feedlot cattle (Swinney-Floyd et al. 1999; Galyean et al. 2000; Rust et al. 2000). Krehbiel et al. (2003) summarized the effects of varying concentrations and strains of *Lactobacillus acidophilus* (LA45 and LA51) and *Propionibacterium freudenreichii* (PF24) on feedlot performance and carcass characteristics of feedlot steers. Steers receiving diets inoculated with DFMs had greater final live weight, average daily gain, hot carcass weight, and carcass average daily gain compared with controls. In their

review, Krehbiel et al. (2003) suggested that feeding a DFM to feedlot cattle would result in a 2.5–5.0% increase in average daily gain and a 2.0% improvement in feed efficiency, although the dry matter intake may be inconsistent.

Ware et al. (1988) reported that feeding *L. acidophilus* BT1386 alone increased the average daily gain and improved feed efficiency in yearling steers fed a high-concentrate diet compared with controls. It did not affect dry matter intake in their experiment. Swinney-Floyd et al. (1999) showed dramatic improvements in average daily gain and feed efficiency when feedlot steers were supplemented with a combination of *L. acidophilus* 53545 and *P. freudenreichii* P-63. During the first 10 days of high-concentrate feeding, average daily gains were 0.93, 1.11, and 1.63 kg/day, respectively, and feed efficiencies were 5.17, 5.32, and 4.50, respectively, for the controls, *P. freudenreichii* alone, and the combination of *P. freudenreichii* and *L. acidophilus*. Across the entire 120-day experiment, feed efficiencies were 5.17, 5.32, and 4.97, and liver abscesses at harvest were 8%, 8%, and 0% for the respective treatments. In the study by Rust et al. (2000), observing the effects of *P. freudenreichii* and two strains of *L. acidophilus* on feedlot steers, cattle receiving a DFM had improved average daily gains, by 6.9%. In the same trial, steers receiving the DFM treatments had improved feed efficiency, by 7.3%, compared to steers on the control treatment (Rust et al. 2000).

McPeake et al. (2002) combined data from six research trials consisting of 1249 steers to summarize the effects of *L. acidophilus* and *P. freudenreichii* on feedlot performance. Contrasts were performed for DFM steers versus control steers. These contrasts revealed greater final live weights, overall average daily gains, and carcass adjusted average daily gains for DFM steers (McPeake et al. 2002). Steers receiving a DFM also tended to have greater overall dry matter intake (McPeake et al. 2002). Cattle receiving a DFM had improved efficiency in a trial evaluating dose titration of *L. acidophilus* combined with a single dose of *P. freudenreichii* (Vasconcelos et al. 2008). However, feed efficiency responded quadratically with increasing doses of *L. acidophilus*, with the lower and higher *L. acidophilus* treatments being numerically greater than the intermediate *L. acidophilus* treatment (Vasconcelos et al. 2008).

In the Vetlife survey regarding DFM usage, it was demonstrated that cattle receiving a DFM did exhibit improved performance (McDonald et al. 2005). Steers receiving a DFM had 1.9% greater average daily gains and demonstrated 1.9% improvement on feed conversion when compared to control steers (McDonald et al. 2005). Heifers on DFMs had 1.4% greater average daily gains and demonstrated 3.9% improvement on feed conversion when compared to control heifers (McDonald et al. 2005).

As previously mentioned, cattle fed bacterial DFMs have demonstrated great variability in dry matter intake. Ghorbani et al. (2002) fed cannulated steers *Propionibacterium* P15 or *Propionibacterium* P15 plus *E. faecium* EF212 and reported no effect of bacterial DFMs on dry matter intake. Others have also reported no effect of bacterial DFMs (*Lactobacillus* and *Propionibacterium*) on dry matter intake in growing cattle (Galyean et al. 2000; Rust et al. 2000; Elam et al. 2003). In contrast, in a summary of experiments, a positive linear effect with increasing *L. acidophilus* was observed for dry matter intake (Krehbiel et al. 2003).

When considering experiments available for review, effects of DFMs on dry matter intake are generally inconclusive.

Although there is considerable evidence demonstrating that bacterial DFMs improve cattle performance, results have been somewhat inconsistent (Krehbiel et al. 2003). This is evidenced by another study of the effects of two strains of *L. acidophilus* combined with a single dose of *P. freudenreichii* (Elam et al. 2003). Elam et al. (2003) determined that the DFMs did not affect animal performance.

9.7   Direct-Fed Microbials and Carcass Merit

In addition to their impact on cattle performance, DFMs have demonstrated the potential to affect carcass characteristics. This impact is generally seen as a yield response causing increases in hot carcass weights while not affecting carcass quality (Krehbiel et al. 2003). A review of the data from six research trials consisting of 1249 cattle by McPeake et al. (2002) showed that *L. acidophilus* and *P. freudenreichii* affected carcass characteristics. This summary confirmed that steers receiving a DFM had greater hot carcass weights when compared to steers receiving a control diet (McPeake et al. 2002). McPeake et al. (2002) observed no significant differences in carcass quality traits for steers receiving a DFM. Most data from DFM research trials suggests that feeding a DFM does not significantly affect the dressing percentage, yield grade, quality grade, or any other carcass traits other than potentially increasing the hot carcass weight (Elam et al. 2003; Krehbiel et al. 2003; Vasconcelos et al. 2008).

9.8   Direct-Fed Microbials to Control the Shedding of *Escherichia coli* O157:H7 in Cattle

The use of DFMs, specifically *Lactobacillus*-based DFMs, to control the shedding of *E. coli* O157:H7 in cattle has received much consideration from both researchers and the cattle industry (Loneragan and Brashears 2005; LeJeune and Wetzel 2007). *Lactobacillus*-based DFMs have repeatedly demonstrated effectiveness in decreasing *E. coli* O157:H7 shedding in cattle (Loneragan and Brashears 2005; LeJeune and Wetzel 2007). In a study evaluating *E. coli* O157:H7 prevalence in feedlot cattle by Brashears et al. (2003), it was discovered that the feeding of *L. acidophilus* NPC 747 decreased *E. coli* O157:H7 shedding in the feces of cattle when compared to the control diet. In addition, supplementation with a DFM decreased the incidence of *E. coli* O157:H7 in the pens and the number of *E. coli* O157:H7-positive hides at harvest (Brashears et al. 2003). These results led Brashears et al. (2003) to suggest that the feeding of *Lactobacillus*-based DFM would decrease fecal shedding of *E. coli* O157:H7 and contamination on hides.

Another trial observed *E. coli* O157:H7 prevalence with various levels of *L. acidophilus* NP51 in combination with *Propionibacterium freudenreichii* (Younts-Dahl et al. 2005). Cattle receiving *L. acidophilus* in combination with *P. freudenreichii* had a lower prevalence of *E. coli* O157:H7 throughout the feeding period, and there was a linear decrease in prevalence with the increasing dose of *L. acidophilus* (Younts-Dahl et al. 2005). These results led Younts-Dahl et al. (2005) to conclude that the feeding of *L. acidophilus* NP51 was an effective preharvest intervention strategy for *E. coli*.

In another study, steers were given various strains of *L. acidophilus* to evaluate the prevalence and enumeration *E. coli* O157:H7 in cattle fed a DFM (Stephens et al. 2007). The prevalence of *E. coli* O157:H7 in control cattle was greater ($P < 0.05$) than in cattle receiving *L. acidophilus* strains NP51, NP28, or NP51-NP35 (Stephens et al. 2007). Tabe et al. (2008) observed that steers receiving an *L. acidophilus* DFM had a significant reduction in fecal shedding of *E. coli* O157:H7 when compared to control steers during the finishing period. he steers on *L. acidophilus* treatment had a 32% decrease in the fecal shedding of *E. coli* O157:H7 (Tabe et al. 2008). Although feeding DFMs has shown inconsistent results, these studies indicate that DFMs have the ability to decrease shedding of *E. coli* O157:H7 in cattle.

9.9 Potential Modes of Action of Direct-Fed Microbials

There are several proposed modes of action for DFMs. The mode of action for a particular DFM can vary with the type of substrate utilized, the feeding strategy employed, the forage-to-concentrate ratio of the diet, and the physiological condition or production consideration of the cattle (Wallace 1994; Lehloenya et al. 2008). There are certain biological conditions that must be met for a DFM to be efficacious and have the mode of action that was intended. The DFM should not be pathogenic; it should be able to survive through all segments of the gut, be specific to the host species, and be a stable organism (Holzapfel et al. 1998). If these biological conditions are met, it has been suggested that DFMs are able to produce organic acids, competitively exclude potentially harmful bacteria, stimulate immune system responses, produce peroxides that have antimicrobial properties, produce enzymes and increase enzyme activity, and reduce toxic amines (Krehbiel et al. 2003; Alliance Animal Health 2009).

Direct-fed microbials exert their effects on the microbial flora of the gut, which could be in the rumen or the small and large intestines. The influence on gut microbes is based on the information derived from changes in the numbers and population types of bacteria in the rumen or the lower gut (contents or feces), changes in fermentation end-products in the rumen or lower gut, decrease in clinical or subclinical infections, and/or increased animal performance (Huber 1997; Nagaraja et al. 1997; Fuller 1999; Brown and Nagaraja 2009). Changes in microbial numbers and population types are often assessed by enumerating bacterial genera or functional groups of bacteria such as lactobacilli, bifidobacteria, or coliforms but most often have failed to detect changes at the species or strain level. However, changes in the metabolic activity and resultant changes in end-products of fermentation have been

measured and are likely more important as they relate to the physiological response of the host (Brown and Nagaraja 2009).

Through the production of organic acids, specifically lactic, acetic, and formic acids, DFMs can inhibit intestinal pathogens or serve as an energy source for other beneficial bacteria and ultimately the animal (Krehbiel et al. 2003; Alliance Animal Health 2009). It has also been suggested that DFMs can competitively exclude other bacteria present in the gut. That is, they could compete with pathogenic bacteria for attachment sites in the intestines and could in turn reduce pathogen loads in the intestine (Salminen et al. 1996; Krehbiel et al. 2003).

Direct fed microbials can stimulate immune system responses. Bacterial DFMs have demonstrated effects on the innate, humoral, and cellular elements of the immune system (Krehbiel et al. 2003). In addition to the GI tract's roles in digestion and absorption of nutrients, it provides a line of defense against the constant presence of antigens in the gut from food and harmful microorganisms (Krehbiel et al. 2003). Certain strains of bacteria have antimicrobial properties. Many species of lactobacilli have been shown to inhibit pathogens (Krehbiel et al. 2003). Lactobacilli have been shown to produce hydrogen peroxide, which demonstrates bactericidal activity (Krehbiel et al. 2003).

Direct fed microbials can also affect enzyme activity in the host animal. Beneficial *Bacillus* spp. produce a wide variety of enzymes including proteases, amylases, lipases, and glycosidases (Alliance Animal Health 2009). DFMs additionally can cause reductions in toxic enzymes in the intestines. Amines produced by some microbes are toxic and have been associated with diarrhea (Alliance Animal Health 2009). Lactic acid bacteria can reduce amine concentrations and neutralize enterotoxins in the gut (Alliance Animal Health 2009).

In addition to these general modes of action, there are targeted modes of action for different types of DFMs or combinations of DFMs. The most well-documented example would be utilizing lactate-producing bacteria such as *Lactobacillus acidophilus* in combination with lactate-utilizing bacteria such as *Propionibacterium freudenreichii* (Raeth-Knight et al. 2007). In this particular example, the presence of the lactate-producing *L. acidophilus* is helping the ruminal microorganisms adapt to the presence of lactic acid (Ghorbani et al. 2002; Beauchemin et al. 2003). The presence of the lactate-utilizing *P. freudenreichii* is helping to prevent lactate from accumulating in the rumen (Kung and Hession 1995; Beauchemin et al. 2003). The intended result of this combination is a decrease in the risk of acidosis and improved feed digestion in feedlot cattle fed a high-grain diet (Beauchemin et al. 2003).

References

AAFCO. 1999. *Official Publication: Association of American Feed Control Officials*, *Inc*. Georgia Department of Agriculture, Plant Food, Feed and Grain Division, Capital Square, Atlanta, GA.

Alliance Animal Health. 2009. *Direct fed microbial: Application and usage*. *Technical Bulletin.* Archer Daniels Midland Company, Decatur, IL. http://www.admani.com/AllianceAnimal Health/Tech%20Bulletins/Direct%20Fed%20Microbials.htm. Accessed March 10, 2010.

Beauchemin, K.A., W.A. Yang, D.P. Morgavi, G.R. Ghorbani, and B. Kautz. 2003. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. *Journal of Animal Science* 81: 1628–1640.

Beauchemin, K.A., C.R. Krehbiel, and C.J. Newbold. 2006. Feed enzymes and direct-fed microbials in ruminant nutrition. In *Biology of nutrition in growing animals*, ed. R. Mosenthin, J. Zentek, and T. Zehrowska. Philadelphia: Elsevier Limited.

Brashears, M.M., M.L. Galyean, G.H. Loneragan, J.E. Mann, and K. Killinger-Mann. 2003. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *Journal of Food Protection* 66: 748–754.

Brown, M., and T.G. Nagaraja. 2009. Direct-fed microbials for growing and finishing cattle. In *Plains Nutrition Council Spring Conference Publication. No. AREC 09–10*, 42–60. Amarillo: Texas A&M Research and Extension Center.

Callaway, T.R., M.A. Carr, T.S. Edrington, R.C. Anderson, and D.J. Nisbet. 2009. Diet, *Escherichia coli* O157:H7, and cattle: A review after 10 years. *Current Issues in Molecular Biology* 11: 67–80.

Elam, N.A. 2003. Effects of live cultures of *Lactobacillus acidophilus* (Strains NP45 and NP 51) and *Propionibacterium freudenreichii* (Strain NP24) on performance, carcass and intestinal characteristics, and *Escherichia coli O157:H7* shedding of finishing beef steers. Ph.D. Dissertation, Texas Tech University, Lubbock, TX.

Elam, N.A., J.F. Gleghorn, J.D. Rivera, M.L. Galyean, P.J. Defoor, M.M. Brashears, and S.M. Younts-Dahl. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. *Journal of Animal Science* 81: 2686–2698.

FDA. 2003. Office of Regulatory Affairs: Compliance Policy Guides. Sec. 689.100 Direct-Fed Microbial Products (CPG 7126.41). http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg689-100.html. Accessed August 10, 2009.

Fuller, R. 1989. A review: Probiotics in man and animals. *The Journal of Applied Bacteriology* 66: 365–378.

Fuller, R. 1999. Modulation of the intestinal microflora by probiotics. In *Probiotics, other nutritional factors and intestinal microflora*, Nutrition Workshop series, vol. 42, ed. L.A. Hanson and R.H. Yolken, 33–45. Philadelphia: Nestle Lte., Veveyl/Lippincott-Raven Publ.

Galyean, M.L., G.A. Nunnery, P.J. Defoor, G.B. Salyer, and C.H. Parsons. 2000. Effects of live cultures of *Lactobacillus acidophilus* (Strains 45 and 51) and *Propionibacterium freudenreichii* PF-24 on performance and carcass characteristics of finishing beef steers. http://www.asft.ttu.edu/burnettcenter/progressreports/bc8.pdf. Accessed June 27, 2002.

Ghorbani, G.R., D.P. Morgavi, K.A. Beauchemin, and J.A.Z. Leedle. 2002. Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. *Journal of Animal Science* 80: 1977–1985.

Gill, D.R., R.A. Smith, and R.L. Ball. 1987. The effect of probiotic feeding on health and performance of newly-arrived stocker calves. Oklahoma agricultural experiment station. *Animal Science Research Report* MP-119: 202–204.

Gilliland, S.E. 1989. Acidophilus milk products: A review of potential benefits to consumers. *Journal of Dairy Science* 72: 2483–2494.

Greening, R.C., W.J. Smolenski, R.L. Bell, K. Barsuhn, M.M. Johnson, and J.A. Robinson. 1991. Effects of inoculation of *Megasphaera elsdenii* strain 407A(UC-12497) on ruminal pH and organic acids in beef cattle. *Journal of Animal Science* 69(Suppl. 1): 518.

Guillen, L.M. 2009. *Determination of the mechanism(s) by which direct-fed microbials control Escherichia coli O157:H7 in cattle*. Ph.D. Dissertation. Stillwater, OK: Oklahoma State Univ.

Holzapfel, W.H., P. Haberer, J. Snel, U. Schillinger, and J.H.J. Huis in't Veld. 1998. Overview of gut flora and probiotics. *International Journal of Food Microbiology* 41: 85–101.

Huber, J.T. 1997. Probiotics in cattle. In *Probiotics 2. Application and practical aspects*, ed. R. Fuller and R. Fuller, 162–186. London: Chapman & Hall.

Huffman, R.P., K.K. Karges, T.J. Klopfenstein, R.A. Stock, R.A. Britton, and L.D. Roth. 1992. The effect of *Lactobacillus acidophilus* on subacute ruminal acidosis. *Journal of Animal Science* 70(Suppl. 1): 87. Abstract.

Kim, S.-W., D.G. Standorf, H. Roman-Rosario, M.T. Yokoyama, and S.R. Rust. 2000. Potential use of *Propionibacterium acidipropionici*, strain DH42, as a direct-fed microbial for cattle. *Journal of Animal Science* 78(Suppl. 1): 292.

Krehbiel, C.R., S.R. Rust, G. Zang, and S.E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *Journal of Animal Science* 81(E. Suppl. 2): E120–E132.

Kung Jr., L., and A.O. Hession. 1995. Preventing in vitro lactate accumulation in ruminal fermentation by inoculation with *Megasphaera elsdenii*. *Journal of Animal Science* 73: 250–256.

Lehloenya, K.V., C.R. Krehbiel, K.J. Mertz, T.G. Rehberger, and L.J. Spicer. 2008. Effects of *Propionibacteria* and yeast culture fed to steers on nutrient intake and site and extent of digestion. *Journal of Dairy Science* 91: 653–662.

LeJeune, J.T., and A.N. Wetzel. 2007. Preharvest control of *Escherichia coli* O157 in cattle. *Journal of Animal Science* 85(E. Suppl): E73–E80.

Lodge, S., T. Klopfenstein, R. Stock, and D. Herold. 1996. Use of direct-fed microbials to alleviate subacute acidosis. *Institute of agriculture and natural resources*, University of Nebraska-Lincoln. Beef Cattle Report MP 66-A, 66–67.

Loneragan, G.H., and M.M. Brashears. 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Science* 71: 72–78.

McDonald, A., P. Anderson, P. DeFoor, and R. Botts. 2005. DFMs improve health, performance of cattle. *Feedstuffs* 77: 12–13.

McPeake, C.A., C.S. Abney, K. Kizilkaya, M.L. Galyean, A.H. Trenkle, J.J. Wagner, D.R. Ware, and S.R. Rust. 2002. Effects of direct-fed microbial products on feedlot performance and carcass characteristics of feedlot steers. *Processes Plains Nutrition. Council, Texas A&M Agricultural Experimental Station Publication. No. AREC 02–02*, 133 (Abstr.)

Metchnikoff, E. 1908. *Prolongation of life*. New York: G.P. Putnam's Sons.

Miles, R.D., and S.M. Bootwalla. 1991. Direct-fed microbials in avian. In *Direct-fed microbials in animal production: A review of literature*, 117. West Des Moines: Nat. Feed Ingred. Assoc.

Nagaraja, T.G., C.J. Newbold, C.J. Van Nevel, and D.I. Demeyer. 1997. Manipulation of ruminal fermentation. Pages 523–632 In: The Rumen Microbial Ecosystem. P.N. Hobson and C.S. Stewart, ed. Blackie Academic and Professional, London.

Nisbet, D.J., and S.A. Martin. 1994. Factors affecting L-lactate utilization by *Selonomonas ruminantium*. *Journal of Animal Science* 72: 1355–1361.

Nocek, J.E., W.P. Kautz, J.A.Z. Leedle, and J.G. Allman. 2002. Ruminal supplementation of direct-fed microbials on diurnal pH variation and in situ digestion in dairy cattle. *Journal of Dairy Science* 85: 429–433.

Owens, F.N., D.S. Secrist, W.J. Hill, and D.R. Gill. 1998. Acidosis in cattle: A review. *Journal of Animal Science* 76: 275–286.

Phillips, W.A., E.E. Grings, and J.W. Holloway. 2005. Effects of a single dose of direct-fed microbials on performance of stocker calves grazing annual cool-season grasses. *The Professional Animal Scientists* 21: 88–92.

Raeth-Knight, M.L., J.G. Linn, and H.G. Jung. 2007. Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of Holstein dairy cows. *Journal of Dairy Science* 90: 1802–1809.

Rust, S.R., K. Metz, and D.R. Ware. 2000. Effects of Bovamine™ rumen culture on the performance and carcass characteristics of feedlot steers. *Michigan Agricultural Experiment Station Beef Cattle, Sheep, and Forage System and Research of the Democratic Republic* 569: 22–26.

Salminen, S., E. Isolauri, and E. Salimen. 1996. Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. *Antonie Leeuwenhoek* 70: 347–358.

Slyter, L.L., R.S. Tung, and L. Kung Jr. 1992. Effect of monensin and laysocellin on growth and fermentation by pure cultures of ruminal bacteria. *Journal of Applied Animal Research* 1: 1–10.

Stephens, T.P., G.H. Loneragan, G.H. Chichester, and M.M. Brashears. 2007. Prevalence and enumeration of *Escherichia coli* O157 in steers receiving various strains of *Lactobacillus*-based direct-fed microbials. *Journal of Food Protection* 70: 1252–1255.

Stern, R.M., and A.B. Storrs. 1975. The rationale of *Lactobacillus acidophilus* in feeding programs for livestock. In *Proc. 36th Minnesota Nutr. Conf.* Bloomington, 191.

Swinney-Floyd, D., B.A. Gardner, F.N. Owens, T. Rehberger, and T. Parrott. 1999. Effect of inoculation with either strain P-63 alone or in combination with *Lactobacillus acidophilus* LA53545 on performance of feedlot cattle. *Journal of Animal Science* 77(Suppl. 1): 77.

Tabe, E.S., J. Oloya, D.K. Doetkott, M.L. Bauer, P.S. Gibbs, and M.L. Khaitsa. 2008. Comparative effect of direct-fed microbials on fecal shedding of *Escherichia coli* O157:H7 and Salmonella in naturally infected feedlot cattle. *Journal of Food Protection* 71: 539–544.

Van Koevering, M.T., F.N. Owens, D.S. Secrist, R.H. Anderson, and R.E. Herman. 1994. Cobactin II for feedlot steers. *Journal of Animal Science* 72(Suppl. 1): 83.

Vasconcelos, J.T., N.A. Elam, M.M. Brashears, and M.L. Galyean. 2008. Effects of increasing dose of live cultures of *Lactobacillus acidophilus* (Strain NP 51) combined with a single dose of *Propionibacterium freudenreichii* (Strain NP 24) on performance and carcass characteristics of finishing beef steers. *Journal of Animal Science* 86: 756–762.

Wallace, R.J. 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: Progress and problems. *Journal of Animal Science* 72: 2992–3003.

Ware, D.R., P.L. Read, and E.T. Manfredi. 1988. Pooled summary of eight feedlot trials evaluating performance and carcass characteristics of steers fed *Lactobacillus acidophilus* strain BT138. *Journal of Animal Science* 66(Suppl. 1): 436.

Yang, W.Z., K.A. Beauchemin, D.D. Vedres, G.R. Ghorbani, D. Colombatto, and D.P. Morgavi. 2004. Effects of direct-fed microbial supplementation on ruminal acidosis, digestibility, and bacterial protein synthesis in continuous culture. *Animal Feed Science and Technology* 114: 179–193.

Yoon, I.K., and M.D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. Asian-Australas. *Journal of Animal Science* 8: 533–555.

Younts-Dahl, S.M., M.L. Galyean, G.H. Loneragan, N.A. Elam, and M.M. Brashears. 2005. Dietary supplementation with *Lactobacillus*- and *Propionibacterium*-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. *Journal of Food Protection* 67: 889–893.

Chapter 10
Future Challenges of Administration of Direct-Fed Microbial Supplementation to Swine

Ellen Davis

Abstract The administration of beneficial bacterial supplements to livestock has been prevalent in the industry for some time for the benefits they provide to animal health and production. These benefits were thought to be derived from competitive exclusion of disease-causing pathogens in the gastrointestinal tract and from the induction of health-promoting bacterial species. The combination of these effects were believed to result in the development of a healthy gastrointestinal microbial ecosystem capable of protecting the animal from disease challenges and the stress of production agriculture, including the stresses of weaning, social commingling, and accelerated growth and production. Recently, our understanding of these benefits has been expanded to incorporate a more detailed understanding of how the administration of beneficial bacteria brings about pathogen inhibition, health, and production performance. This chapter addresses our more recent understanding of direct-fed microbial benefits when provided to swine and how this knowledge has illuminated how much more is needed to harness the potential direct-fed microbials have as a tool for reaching swine's genetic potential for efficient production.

10.1   Introduction

The administration of beneficial bacterial supplements to livestock has been prevalent in the industry for some time for the benefits they provide to animal health and consequently, to efficiency of meat, milk, and egg production. These benefits were perceived to be derived from competitive exclusion of disease-causing pathogens in

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the gastrointestinal (GI) tract and from the induction of health-promoting bacterial species. The combination of these effects were thought to result in the development of a healthy GI microbial ecosystem capable of protecting the animal from disease challenges and the stress of production agriculture, including the stresses of weaning, social commingling, and accelerated growth and production. Recently, our understanding of these benefits has been expanded to incorporate a more detailed understanding of how the administration of beneficial bacteria brings about pathogen inhibition, health, and production performance. This chapter addresses our recent understanding of direct-fed microbial (DFM) benefits when provided to swine and how this knowledge has illuminated how much more is needed to harness the potential of DFMs as a tool for reaching swine's genetic potential for efficient production. These gaps in our knowledge of how the GI microbial consortia interact with host physiology and how these interactions can be strategically manipulated to get around the current limitations in deriving the most benefit to swine production from the administration of DFMs.

10.2 Influence of Swine Management Practices on the Gastrointestinal Microbiota

Common management practices implemented throughout swine production phases affect the development of the microbial consortia. Early microbial exposure and the weaning event likely result in the most dramatic influences in the GI microbiota. Thompson et al. (2008) reported that the microbial diversity in neonatal piglets may be associated with the interaction of random microbial exposure from the rearing environment and the variability of genetic expression within the individual, emphasizing the importance of early life environment on microbial colonization and its impact on phenotypic expression of genetic potential. Microbial changes in response to the weaning event were reported by Konstantinov and coworkers (2006), who found that the predominant microbial species present in the ileum prior to weaning at 3 weeks of age were *Lactobacillus sobrius* and *L. reuteri*. Yet soon after abruptly weaning the piglets from the sow, these predominating *Lactobacillus* populations were greatly reduced, and clostridia and *Escherichia coli* had emerged as the predominant members of the microbiota in the ileum. The question that arises from the documentation of this shift in the microbial consortia resulting from abruptly weaning the piglet from the sow is what impact this specific change in the GI microbial population has on the future productive performance of the piglet in later growth stages. Furthermore, how does this microbial shift differ from gradual weaning from the sow or weaning the pig at a different development stage (i.e., at an earlier or older age)?

Evidence of the influence that management and environment have on microbial development at an early age and subsequently in later adult life was demonstrated by the investigation of the early life environment and its influence on subsequent microbial diversity present in adults in a pig model of indoor, outdoor, and environmentally

isolated management conditions (Mulder et al. 2009). In this study, pigs reared in the outdoor environment exhibited lower microbial diversity in the GI tract during adulthood compared to those reared in the more hygienic indoor and environmentally isolated rearing conditions. Although the outdoor reared pigs were exposed to greater microbial diversity in their environment during early and later life, their microbial diversity exhibited an increase in the Firmicutes phylum compared to the other two environments, made up primarily of beneficial lactobacilli. Furthermore, regulation of gene expression of numerous cytokines and chemokines in the indoor and environmentally isolated pigs indicated immune activation in the gut environment, whereas the outdoor reared pigs exhibited a lack of proinflammatory immune expression indicative of GI immune-tolerant and homeostatic responses. Previous thinking emphasized the importance of the rich diversity in the GI tract for protection against dramatic shifts in the gut microbiota and maintenance of consistency within the gut microbial ecosystem (Backhed et al. 2005). The work of Mulder et al. (2009), however, indicates that early exposure to a microbially diverse environment predisposes colonization by a limited number of phyla dominated by microorganisms that likely provide health-promoting benefits. This suggests more importance is placed on the specific members that make up the dominant consortia than the diversity in the intestinal microbial ecosystem.

Other studies have investigated how specific members of the microbial consortia differ between pigs reared in diverse management environments at an early age. Specifically, subsequent pig health and performance was associated with the presence of distinct terminal restriction fragments from genomic DNA of microorganisms present in the GI tracts of pigs reared in segregated weaning facilities compared to weaning to on-site nursery facilities (Davis et al. 2010a). Terminal restriction fragment length polymorphism analysis revealed peaks with base lengths putatively identified as *Lactobacillus* sp. and a specific strain of *Pediococcus acidilactici* that was identified using 16S rRNA gene sequencing whose presence in the intestinal tract was positively correlated to pigs reared in the segregated weaning system. Furthermore, immune cell populations identified with flow cytometric analysis were determined to be associated with the two weaning management environments. Specifically, γδ T-cell populations in the jejunal intraepithelial compartment were positively correlated with conventional management, whereas activated cytotoxic T cells and T cells with memory phenotypes were positively correlated with segregated weaning. Immunohistochemical analysis of jejunal samples further revealed drastic fluctuations in cytotoxic (CD8+) and T-helper (CD4+) cell populations between the two nursery management conditions relative to days after weaning (Brown et al. 2006a, b). As management was the only factor differing between these groups of pigs, these data support the premise that distinct microbial populations emerged in these groups that were reared in distinct management and environmental conditions that resulted in divergent immune development relative to the number of days after the weaning event.

The effect of early environmental exposure and microbial colonization of the piglet and its effect on subsequent growth performance was further demonstrated when intestinal microbial communities and immune cell phenotypes were investigated in

pigs farrowed and reared in conventional farrowing facilities compared to outdoor farrowing facilities and weaned to indoor, environmentally controlled segregated weaning facilities. Although the two groups of pigs were housed and managed identically during the nursery production phase, pigs that had previously been reared in the outdoor management system weighed 0.5 kg less than pigs reared in confinement farrowing facilities at weaning but weighed 2 kg more 42 days later at the end of the nursery phase (Davis et al. 2009a, b). This separation in growth performance response during the nursery phase between pigs previously reared in distinctly different environments, both conventional versus segregated weaning management and indoor versus outdoor farrowing management, allowed relationships to be investigated in the importance of the divergent microbial communities and immune development established in the pigs to subsequent growth response (Rehberger et al. 2009; Davis et al. 2010b). Terminal restriction fragments (TRFs) identified through 16S gene sequencing as strains of *Lactobacillus acidophilus*, *L. salivarius*, and *Pediococcus acidilactici* had the strongest associations with the segregated nursery management system and the outdoor rearing environment, and these bacterial TRFs also had strong positive correlations with pig growth performance measures.

A specific strain (strain PAL) identified as *Pediococcus acidilactici* was isolated from outdoor pigs with TRFs that were positively correlated with body weight gain as well as several immunological measurements. Specifically, this strain of *P. acidilactici* identified as having these TRFs was associated with T cells expressing the γδ T-cell receptor and T cells with cell surface markers indicative of a memory cell phenotype in the jejuna intraepithelial compartment and in the systemic circulation (Davis et al. 2010b). The identification of specific immune cell phenotypes in pigs exhibiting the greatest growth performance responses provides a means of identifying specific immune factors associated with pigs having the greatest body weight after weaning (Davis et al. 2009a, b). Cell phenotypes consisting of T cells expressing γδ T-cell receptors and leukocytes expressing the interleukin-2 (IL-2) receptor indicative of an activated population were associated with pigs having the greatest body weight within the first 2 weeks after weaning. Other research has identified that proportions of lymphocyte phenotypes expressing cell surface markers indicative of natural killer cells, T-helper and cytotoxic T cells, and major histocompatibility complex (MHC) II in the peripheral blood were negatively associated with body weight gain, feed intake, feed efficiency, and carcass characteristics in pigs from birth to market weight (Galina-Pattoja et al. 2006).

These data just begin to illuminate the complex nature of the interactions between early environmental exposure, colonization of a complex gut microbial ecosystem, immune development in which responses are orchestrated to promote whole organism homeostasis, and ultimately the realization of efficient pork production. Although differences in the microbiota were identified between pigs reared in diverse environments during early life development and divergent immune populations related to members of the microbial consortia and to phenotypic measures of pig growth performance, the discovery of the most beneficial microbial contributors, the crucial time points when these contributors need to be present, and other interactions with yet unidentified factors that are needed to be able to strategically

dictate the microbial ecosystem development for optimal responses in later pig production phases is still a challenging void.

10.3 Current Administration of Direct-Fed Microbials and Limitations

Many of the microbial strains that are used as DFMs for swine and other livestock species have been selected based on their efficacy in food applications, their capacity to demonstrate the potential to thrive in the GI tract (acid and bile tolerance), and/or ability to maintain viability and stability when administered in animal feed. Less consideration has been made when selecting appropriate microorganisms to provide DFM supplements to meet the specific needs of pigs at various growth stages, challenge environments, and management practices. Yet, a more complete understanding of microbial succession in the GI tract of the neonatal pig and the ramifications the presence of these microbial populations at specific developmental stages and environments in the pig's production life have on subsequent growth and production is crucial to glean the most benefit from strategic application of beneficial microbial strains to promote efficient pork production. Although a complete understanding is lacking, recent data evaluating DFMs administered to pigs has surfaced that provide some insight into how these microbial representatives may be affecting pig health and performance.

The exposure of pigs either directly or indirectly to a specific DFM strain or combination of strains affects the GI microbial consortia, immune development, and pig growth response. Therefore, consideration should be given to whether DFMs must be administered directly to the neonatal piglet or benefits to the piglet can be derived from administering the DFM to the sow. The direct administration of a combination of *Lactobacillus amylovorus* and *Enterococcus facium* bacteria to 8-kg weaned pigs through daily oral gavage resulted in improved feed efficiency, decreased fecal enterobacteria, and a decrease in intestinal immune cell infiltration (Ross et al. 2010). Similarly, supplementation of *Lactobacillus brevis* strain 1E1 to piglets during the neonatal and early postweaning periods in a milk supplement and watering lines, respectively, resulted in the induction of a unique band in the jejunum identified as unculturable, low guanine-cytosine (G/C) Gram-positive bacteria by denaturing gradient gel electrophoresis (DGGE) (Davis, et al. 2007).

Unquestionably, the direct administration of DFMs has the ability to have a positive impact on growth, the GI microbiota, and immune characteristics in the young pig. Yet, the commensal microbiota of the neonatal pig is naturally colonized through exposure to microorganisms from the immediate environment. Contact with the sow and her fecal microbiota likely has a profound influence on the piglets' early exposure to the microbial contributors of the microbial consortia. This indicates that administration of DFMs to the sow would influence the microbial environment to which pigs are exposed by inoculation with the DFM organisms or altering the GI microbial populations in the sow. When a combination of *Bacillus*
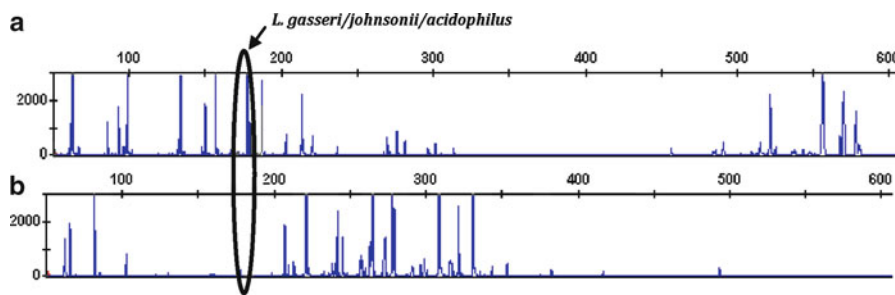
Fig. 10.1 Chromatograms illustrate the presence of a terminal restriction fragment peak resulting from digestion with MspI restriction enzyme at 187 base pairs putatively identified as *Lactobacillus gasseri/johnsonii/acidophilus* present in the colon of piglets at 3 days of age born to sows supplemented with a *Bacillus*-based direct-fed microbial (**a**) and the absence of this peak in 3-day-old piglets born to unsupplemented sows (**b**) (Described by Baker et al. 2010)

licheniformis and *B. subtilis* strains were administered to sows during the late gestation and lactation periods, their piglets demonstrated decreased preweaning mortality and increased body weight at weaning compared to piglets nursing unsupplemented sows (Alexopoulos et al. 2004). Baker et al. (2010) demonstrated that administration of a *B. subtilis*-based DFM to the sow shifted the GI microbiota of the piglets toward a greater prevalence of *Lactobacillus* sp. compared to pigs in litters from unsupplemented sows, which conversely had a greater prevalence of *Clostridium* sp. in the GI tract compared to pigs from treated sows. Furthermore, a specific species putatively identified as *Lactobacillus gasseri* was identified through terminal restriction fragment length polymorphism analysis and was determined to be consistently present in the small intestine of pigs from DFM-treated sows and absent in the GI tracts of pigs from unsupplemented sows (Fig. 10.1). Whether it was this change resulting from alteration of the sow's microbial community that changed the early microbial exposure of the neonatal pig from the environment, or the DFM was directly transferred to the piglets through fecal-oral transfer from shedding of the DFM organisms by the sow, remains uncertain. These data indicate that inoculation of the sow with a DFM can have profound effects on early colonization of the commensal microbiota in the neonatal piglet.

These data illustrate the potential of DFM administration as a tool to strategically guide the development of early microbial colonization, emphasizing the importance of supplying the appropriate DFM relative to the pig's production stage. It is conceivable that immediate benefits from DFM supplementation may not be immediately evident but, instead, benefits may manifest during later stages of production. Administration of *Lactobacillus brevis* strain 1E1 via a milk supplement to neonatal pigs nursing sows resulted in improved pig growth performance during the nursery period, with no difference between treated and untreated pigs during the preweaning period (Brown et al. 2003). Although an improvement in growth response was not evident during the time that the direct-fed microbial was administered, the number of CD4+ T-helper lymphocytes was lower at weaning in the jejunum of pigs

administered *L. brevis* compared to untreated pigs, and gene expression of toll-like receptor 4 (TLR4) and TLR9 as well as several adaptor proteins in the toll-like receptor signaling pathway were reduced with *L. brevis* treatment (Brown et al. 2006a, b; Halbrook et al. 2005).

These studies demonstrate that administration of DFMs during early postnatal development in the young pig can influence the composition of the commensal microbiota, coordinate immune development, and translate into improved growth performance during the time when the DFM was administered and during subsequent growing phases in swine production. The greatest gap in the collective knowledge is how alteration of the members of the GI microbial community and immune development translates into improved growth and efficiency. Likely, there are specific desirable microbial contributors in the GI tract that relate to dietary, health, genetic, and management conditions; and ascertaining how the microbial population can be manipulated to meet these conditions is the next frontier of discovery for furthering the effective use of DFM supplementation in pigs.

10.4 Host–Microbial Interactions

Further understanding of the implications of changes to the GI microbiota and how these changes relate to pig immune development, health, and growth performance is a key challenge for gleaning the most benefit from DFM supplementation. The host–microbe interaction between the GI microbiota and the intestinal mucosal surface has been implicated as the obvious site of host–microbe communication to establish homeostatic responses by the host to the commensal microbial population. This cellular and molecular cross talk between members of the microbial consortia, intestinal epithelial cells, immune cells of the GI tract, and pattern recognition receptors on host cell surfaces such as toll-like and NOD receptors has been nicely reviewed by Winkler et al. (2007), and the expansion of our understanding of this communication between host and enteric microbiota has furthered the concept of the breadth of influence that the intestinal bacterial population has on the host.

Various microorganisms influence the immune system to induce divergent immune development patterns. For instance, microbial populations in the GI tract guide immune development in the young animal and have the capacity to influence immune differentiation to the inflammatory T-helper lymphocyte type 1 (Th1), the antibody-promoting Th2, or regulatory Th3 T-cell subsets, functionally simplifying these T-cell immune categories. Specifically, certain cells of the immune system in the GI tract function to sample the luminal contents and signal to the host how to respond to the luminal environment (Hord 2008). Immune and epithelial cell sampling and signaling via toll-like and NOD-like receptors are responsible for homeostasis and regulation of appropriate host responses to harmful pathogens and harmless environmental antigens. Even less well understood is how the programming of the host response by the gut microbiota affects metabolic activities, nutrient acquisition from dietary components, and subsequently animal phenotypic growth responses.

Studies with conventional and germ-free mice have provided some knowledge on the potential of the GI microbiota to influence nutrient metabolism of the host. Wikoff et al. (2009) reported that conventional mice have much lower concentrations of plasma tryptophan due to the expression of tryptophanase by specific microbial members of the enteric microbiota, which converts tryptophan to indole, pyruvate, and ammonia, indicating that the commensal microbiota influence dietary amino acid acquisition. The composition of the GI microbiota has also been related to the level of energy that the host can derive from dietary sources. For instance, an increase in the *Firmicutes/Bactertoidetes* ratio was associated with obese humans and mice consuming the same diet as their lean counterparts (Ley et al. 2005, 2006). This finding illustrates the importance of specific enteric microbial populations on amino acid metabolism and brings in to question how multiple bacterial species combinations present in the intestinal ecosystem influence the metabolism of dietary nutrients.

Although the GI tract is usually associated with nutrient acquisition and metabolism and is often mentioned as the location of the largest concentration of immune cells and hence is the largest lymphoid organ, it is important to consider that the GI tract has a vast network of neurons for bidirectional communication between the brain and the intestinal tract. The GI microbiota in concert with the entire luminal contents of the intestine provide stimuli that interact with the enteric nervous system. Signals from these stimuli are influential on intestinal motility and feed intake by afferent neural signaling and neurotransmitters such as cholecystokinin and glucagon (Bienenstock et al. 2010; Hord 2008; Moran 2009). Related to feed intake but less studied is the influence the GI microbiota, immune inflammation, and neural signaling have on behavioral responses of the host. For instance, the stress response has been implicated in altering the microbial ecology of the GI tract, and the composition of the microbial consortia has been related to behavioral responses and disorders (McLean et al. 2009). Unlocking the intricacies of these complex interactions has obvious ramifications for the potential to exploit DFM supplementation to influence efficient nutrient utilization, behavioral responses such as those related to feed intake, and desirable carcass characteristics for meat production in swine.

10.5   Conclusion and Future Directions

The advent of new molecular techniques has already begun to aid in our understanding of how the host and microbial consortia interact, and this information will provide future insight into strategic application of DFMs. Specifically, our challenge is to identify bacterial strains that elicit defined responses in the host such that the future of DFM supplementation to swine diets can be tailored to provide the most benefit to the pig at various production stages or administered as a prescription to address the specific needs of a swine production system. Imagine such a complete understanding of how management, animal genetics, feedstuffs, environment, and the host intestinal microbial ecology interact that a swine production system could

have DFM strains administered during each phase of production for optimal pork production efficiency. Expansion of knowledge and innovation in this area is the fundamental challenge for gleaning the most benefit from future DFM supplementation in swine and other livestock species.

References

Alexopoulos, C., I.E. Georgoulakis, A. Tzivara, et al. 2004. Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores, on the health status and performance of sows and their piglets. *Journal of Animal Physiology and Animal Nutrition* 88: 381–392.

Backhed, F., R.E. Ley, J.L. Sonnenburg, et al. 2005. Host–bacterial mutualism in the human intestine. *Science* 307: 1915–1920.

Baker, A., E. Davis, and T. Rehberger. 2010. Characterization of the gastrointestinal microbiota in neonatal pigs from sows supplemented with a *Bacillus*-based direct fed microbial. *Journal of Animal Science* 88(Suppl. 2): 387.

Bienenstock, J., P. Forsythe, K. Karimi, et al. 2010. Neuroimmune aspects of food intake. *International Dairy Journal* 20: 253–258.

Brown, D.C., M.E. Davis, C.V. Maxwell, et al. 2003. Effect of milk supplementation with *Lactobacillus brevis* 1E-1 on intestinal microflora, intestinal morphology, and pig performance. *Journal of Animal Science* 81(Suppl.2): 76.

Brown, D.C., M.E. Davis, B. Lockhart, et al. 2006a. Effect of milk supplementation with *Lactobacillus brevis* 1E1 on jejunal gene expression. In *Poster presented at the Experimental Biology 2006 meeting in San Fransisco*, 1–5 April 2006, FASEB abst#668.16.

Brown, D.C., C.V. Maxwell, and G.F. Erf. 2006b. The influence of different management systems and age on intestinal morphology, immune cell numbers, and mucin production from goblet cells in post-weaning pigs. *Veterinary Immunology Immunopathology* 111: 187–198.

Davis, M.E., D.C. Brown, A. Baker, et al. 2007. Effect of direct-fed microbial and antibiotic supplementation on gastrointestinal microflora, mucin histochemical characterization, and immune populations of weanling pigs. *Livestock Science* 108: 249–253.

Davis, E., C.V. Maxwell, J.D. Spencer, et al. 2009a. Growth performance of pigs farrowed in indoor verses outdoor management systems and weaned into an indoor, off-site nursery. *Journal of Animal Science* 87(E-Suppl. 3): 88.

Davis, E., J. Rehberger, M. King, et al. 2009b. Peripheral blood and jejuna intraepithelial immune cell phenotypes associated with body weight of newly weaned pigs. *Journal of Immunology* 182: 81.9. abst.

Davis, E., J. Rehberger, M. King, et al. 2010a. Characterization of gastrointestinal microbial and immune populations post-weaning in conventionally-reared and segregated early weaned pigs. *Livestock Science* 133: 92–94.

Davis, E., J. Rehberger, M. King, et al. 2010b. Association of systemic and intestinal immune populations and growth performance traits with terminal restriction fragments defined as *Pediococcus acidilactici* strain PAL in young swine. In *Proceedings of the international conference on probiotics and prebiotics*, Kosice, 15–17 June 2010.

Galina-Pattoja, L., M.A. Mellencamp, J. Bastiaansen, et al. 2006. Relationship between immune cell phenotypes and pig growth in a commercial farm. *Animal Biotechnology* 17: 81–98.

Halbrook, E.A., C.V. Maxwell, D.C. Brown, et al. 2005. Effect of milk supplementation with *Lactobacillus brevis* 1E1 on immune cell numbers in the small intestine of piglets. *Journal of Animal Science* 83(Suppl. 1): 33.

Hord, N.G. 2008. Eukaryotic-microbiota crosstalk: Potential mechanisms for health benefits of prebiotics and probiotics. *Annual Review of Nutrition* 28: 215–231.

Konstantinov, S.R., A.A. Awati, B.A. Williams, et al. 2006. Post-natal development of the porcine microbiota composition and activities. *Environmental Microbiology* 8(7): 1191–1199.

Ley, R.E., F. Backhed, P. Turnbaugh, et al. 2005. Obesity alters gut microbial ecology. *Proceedings of the National Academy Science* 102: 11070–11075.

Ley, R.E., P.J. Turnbaugh, S. Klein, et al. 2006. Microbial ecology: Human gut microbes associated with obesity. *Nature* 444: 1022–1023.

McLean, P.G., A.R. Calver, D.H. Alpers, et al. 2009. The emerging role of the microbial-gastrointestinal-neural axis. *Gastroenterology Insights* 1: e3.

Moran, T.H. 2009. Gastrointestinal signals: Satiety. In *Encyclopedia of neuroscience*, ed. L.R. Squire. Amsterdam: Elsevier.

Mulder, I.E., B. Schmidt, C.R. Stokes, et al. 2009. Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. *BMC Biology* 7: 79–98.

Rehberger, J., E. Davis, M. King, et al. 2009. The effect of management practice on the commensal microbiota of the weanling pig. In *Poster presented at the 109th general meeting of the American society of microbiology in Philadelphia*, 17–21 May 2009.

Ross, G.R., C. Gusils, R. Oliszewski, et al. 2010. Effects of probiotic administration in swine. *Journal of Bioscience Bioengineering* 109(6): 545–549.

Thompson, C.L., B. Wang, and A.J. Holmes. 2008. The immediate environment during postnatal development has long-term impact on gut community structure in pigs. *The ISME Journal* 2: 739–748.

Wikoff, W.R., A.T. Anfora, J. Liu, et al. 2009. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of the United States of America* 106(10): 3698–3703.

Winkler, P., D. Ghadimi, J. Schezenmeir, and J.P. Kraehenbuhl. 2007. Molecular and cellular basis of microflora–host interactions. *Journal of Nutrition* 137: 756S–772S.

Chapter 11
Characteristics and Modification of the Intestinal Tract Microbiota of the Channel Catfish *Ictalurus punctatus*

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Abstract Catfish is the leading commercial aquaculture enterprise in the United States, and many of the issues that have been important for other mass-produced food animals have become areas of research focus for potential improvement in the growth performance and health of catfish. A critical component that can influence both the health and nutrition of catfish is the intestinal tract. The intestinal tract, in addition to being the point of origin for digestion and absorption of nutrients derived from consumed diets, harbors an indigenous microflora that can interact with the host. The composition and role of the intestinal microbial communities in fishes remain poorly understood. To understand the effects of the entire microbial community on the host, additional studies and improved isolation methods are recommended. Along with the lack of knowledge about the composition of the community, little is known about the role of these microorganisms in the intestinal tract. An increased understanding of the intestinal microflora in catfish has potential for manipulation or alteration to improve disease resistance and growth performance, allowing the channel catfish to consume diets made with less expensive ingredients, such as crop residues, already fairly cheap. Recent aquaculture feed trials using prebiotics and probiotics report enhanced physiological and immune responses that contribute to improvements in aquaculture health. These trials are reviewed.

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11.1   Introduction

The channel catfish (*Ictalurus punctatus*) is one of the many important commercial fishes in North America. Channel catfish have been used in commercial aquaculture because they consume a wide variety of food sources, are efficient at conversion of food to body mass, and are adaptable to environments commonly used in large-scale aquaculture. Catfish farming, on average, accounts for 46% of all U.S. aquaculture sales (Mississippi Agricultural and Forestry Experiment Station, Mississippi State University Extension Service 2010).

Many of the issues that have been historically important for other mass-produced food animals have become areas of research for potential improvement in the growth performance and health of commercial catfish aquaculture. A critical component that can influence the health and nutrition of catfish is the intestinal tract. In addition to being the point of origin for digestion and absorption of nutrients derived from consumed diets, the intestinal tract harbors an indigenous microflora that can interact with the catfish host. Intestinal microflora have been studied extensively in other food animals and can vary in their importance to the host. For mammalian ruminants such as cattle and sheep, the rumen microflora represents an essential symbiotic relationship that allows these animals to survive as primary herbivores (Odenyo et al. 1994; Collado and Sanz 2007). For nonruminant animals such as swine and chickens the nutritional benefits are less clear, but at the very least the intestinal microflora serves as a barrier to organisms that may be pathogenic to the host (Nisbet 2002; Ricke and Pillai 1999). This review describes the microbial community of the intestinal tract of catfish and discusses whether the microbiota, by virtue of its makeup and metabolic capabilities, might have an influence on nutrition of the catfish. In addition, the potential for manipulation of the intestinal microflora is examined as are strategies for optimizing microflora composition to benefit the host.

11.1.1   *Ecology and Habitats of Ictalurus Punctatus*

Channel catfish are native from Montana eastward to the Ohio Valley and southward through the Mississippi Valley to the Gulf of Mexico and into Florida. Channel catfish are also native to Mexico (Jordan and Evermann 1908; Eddy and Underhill 1974). However, through introduction, channel catfish now inhabit many eastern and far western rivers distant from their original range (Walden 1964). The original habitat of channel catfish was relatively clear, moderate to swiftly flowing streams (Walden 1964). However, catfish are now frequently found in mud-bottomed, sluggish rivers and creeks as well as weedy ponds and lakes (Jordan and Evermann 1908; Bailey and Harrison 1948; Walden 1964; Eddy and Underhill 1974). Channel catfish have readily adapted to this extreme change in habitat. In fact, channel catfish grow well in diverse natural aquatic habitats, including those with low levels of dissolved oxygen (DO) and relatively elevated temperatures (Moyle and Cech 1988).

The body of the channel catfish is light-brown to slate-gray and a darker gray-brown on top. However, some catfish have a dark brown or olive-green color with light yellow or grayish-green sides and a dirty white underside. Channel catfish can be distinguished from other species in the genus by the presence of spots on its sides (Walden 1964). The head is pointed and small, with a large, terminal mouth (Federal Coordinating Council on Science, Engineering and Technology 1983). The upper jaw slightly overhangs the lower jaw with pads of fine, sharp teeth in the upper jaw (Forbes 1888; Eddy and Underhill 1974). These teeth allow the catfish to grasp and hold both soft and hard prey items. The pharyngeal jaws have small, pointed denticles on both the upper and lower jaws, allowing the catfish to crush insects and mollusk shells and to grind plant material in the diet (Forbes 1888). The caudal fin is forked and the anal fin is long, wide and rounded, supported by 25–30 rays.

Channel catfish tend to be nocturnal and feed primarily on the bottom of rivers and lakes (Burch 1970; Rohde and Arndt 1994). Seasonal changes in the food habits of channel catfish are usually minimal (Bailey and Harrison 1948). The channel catfish is omnivorous, consuming a wide range of foods, including elm seeds, terrestrial and aquatic insects, crayfish, small fish, and aquatic vegetation (Forbes 1888; Bailey and Harrison 1948; Walden 1964; Rohde and Arndt 1994). Insects are the principal food of the channel catfish; however, it has been found that one-fourth of the food consumed by these fish is plant material, mainly algae (Forbes 1888). In addition to plants and animals, channel catfish consume detritus. For this reason, the catfish is often considered a scavenger and "cleaner" of lakes and rivers (Walden 1964).

11.2 *Ictalurus punctatus* Commercial Production

11.2.1 *Aquaculture*

Most catfish used in aquaculture are reared in earthen ponds, although some may be raised in cages or tanks. Conditions in these artificial habitats, such as DO, and temperature influence the growth rates of the fish. Oxygen in the water is produced by algae and may be derived from oxygen diffusion from air. A portion of the oxygen is used by the catfish; however, approximately 80% of the oxygen is used by the algae and other pond organisms (Dunning 1995). Active photosynthesis and respiration of these abundant planktonic organisms can significantly change the oxygen concentrations over short periods of time. Consequently, the catfish must be tolerant of low oxygen conditions to survive. In addition, catfish grow most rapidly at an optimum temperature range of 26–30°C. Feeding activity slows when channel catfish are exposed to temperatures above or below the optimum range (Andrews and Stickney 1972). The solubility of DO in water is temperature-dependent, and the amount of DO at saturation decreases as the temperature increases. This inverse relation exists because organisms tend to respire more as the temperature increases; therefore, DO depletion is greatest during periods of high biological oxygen demand. The DO in culture ponds may fluctuate between 15 mg/L in the afternoon to ≤3 mg/L

at dawn (Carter and Allen 1976). However, channel catfish are somewhat tolerant of lower levels of DO. Therefore, aquaculturists accept a DO concentration of 5.0 mg/L as sufficient to support optimum growth of some fishes because levels below this concentration inhibit energy-requiring metabolic activities, such as growth and reproduction (Carter and Allen 1976; Moyle and Cech 1988).

11.2.2 Nutrition and Commercial Feeding Regimes

Along with DO and temperature, growth rates are largely dependent on the nutrition of the fish (Bailey and Harrison 1948). Catfish feeds are formulated to contain three primary nutritional components: protein, carbohydrates, and lipids. Most feeds also contain a mix of vitamins and minerals. The most expensive component of a catfish diet is protein, the amino acid source. Protein in the feed comes from plant-based or animal sources (Hepher 1988; Robinson et al. 2001). Fish meal is the highest quality protein source available to fish feed manufacturers. However, because of the high costs associated with obtaining fish meal, it is generally used sparingly in commercial production fish feeds (Lovell 1989; Goddard 1996; Robinson et al. 2001). Therefore, production feeds depend primarily on soybean meal and cottonseed meal for protein (Hepher 1988; Lovell 1989; Goddard 1996). Data for amino acid availability in different protein sources is limited, although feed formulations should be based on amino acid availability, not the amount of digestible protein (Robinson et al. 2001). Commercial feed typically contains 28–38% protein (Burch 1970; Stickney 1993; Dunning 1995). Carbohydrates, primarily in the form of starch, comprise up to 40% of the feed, and approximately 6% of the feed is lipids. The remainder of the feed is typically composed of vitamins, minerals, and fiber (Stickney 1993).

Commercial catfish require cost-effective feed. Feed represents the highest variable cost associated with commercial catfish farming, accounting for as much as 50–60% of annual operating costs (Dunning 1995; Robinson et al. 2001). It has been suggested that certain components of the diet can be substituted with less expensive plant material. Modern catfish feeds are comprised of relatively few ingredients, although price can vary based on fluctuations in the price of feed stuffs used in the feeds and in the costs of energy associated with producing these feeds (Stickney 1993; Naylor et al. 2009). Fish nutritionists have tried to use less expensive plant proteins, such as soybean meal or distillers' grains with solubles (DGS), to replace fish meal partially or totally (Hastings and Dickie 1972; Webster et al. 1992; Lim et al. 2009). Webster et al. (1992) found that a diet with 0% fish meal, 35% DGS, and 49% soybean meal can support weight gains in channel catfish similar to those achieved with diets containing high percentages of fish meal. Lim et al. (2009) found that diets in which soybean meal and corn meal were replaced with DGS supplemented with lysine resulted in comparable growth responses as well as improved immune responses in channel catfish.

Both the adverse and beneficial effects of fish meal substitutes should be considered when formulating feeds. Phytoestrogens contained in soybean meal were shown

to affect sex differentiation, changing the sex ratios of populations of farm-raised channel catfish (Green and Kelly 2009). The role of the fish intestinal microflora in response to dietary changes needs to be determined. Understanding the microbial response may lead to better predictions on which dietary combinations are most likely to yield consistently optimal responses. The ecology and characteristics of fish gastrointestinal (GI) microflora are discussed in the following sections.

11.3   Ecology of the Intestinal Microbiota

11.3.1   Diversity of Microflora in Intestinal Tracts of Other Eukaryotic Organisms

The intestinal microbial communities of organisms as diverse as termites, ruminants, chickens, and humans have been characterized (Hungate 1966; Moore and Holdeman 1974; Baldwin and Allison 1983; Odenyo et al. 1994; Prescott et al. 1996; Malinen et al. 2005; Collado and Sanz 2007; Rehman et al. 2007; Wertz and Breznak 2007). In the termites, ruminants, and humans examined, the microbial communities have been found to be comprised of complex assemblages of up to 300 or more species, most of which are obligately anaerobic bacteria (Baldwin and Allison 1983; Breznak 1984; Odenyo et al. 1994; Prescott et al. 1996; Shi et al. 1997; Shi and Weimer 1997; Malinen et al. 2005; Collado and Sanz 2007; Wertz and Breznak 2007). An additional general characteristic of these communities is that species composition appears to vary significantly depending on the host. For example, in the phylogenetically "lower" termites, the intestinal microbiota includes bacteria as well as protozoans. However, in the "higher" termites, the gut is inhabited almost entirely by bacteria. Most bacteria isolated from termites and other insects, such as leaf-cutter ants, are facultative and obligate anaerobes, including strains of *Streptococcus*, *Bacteroides*, various Enterobacteriaceae, *Staphylococcus*, and *Bacillus* (Breznak 1984; Brauman et al. 2001; Noda et al. 2009; Pinto-Tomas et al. 2010; Strassert et al. 2010).

11.3.2   Fish Intestinal Tract Anatomy

Although the diversity of the microbial community of termites, ruminants, and humans can reflect the diet of the host, it may also be a reflection of differences in intestinal tract anatomy. One major difference between the digestive tract of ruminants and fishes is the difference in gut morphology. Ruminants have a highly specialized fermentation chamber with four compartments. The reticulum is small, but the opening into the rumen is large, which makes the reticulum resemble an anterior pouch of the rumen. The reticulum is separated from the rumen by a ridge called the

ruminoreticular fold (Hungate 1966). In nonruminant mammals such as horses, pigs, and rabbits, fermentation occurs in an enlarged cecum (Hungate 1966; Atlas and Bartha 1993). The stomach, though not divided, shows an esophageal groove and compartmentation. In domestic poultry paired ceca are 15–18 cm in length, blind pouches consisting of a narrow constricted open end connected to the colon and a dilated thinner-walled blind component (McNab 1973).

In contrast to the complex anatomy of mammalian herbivores, most fish have an uncomplicated gut. They essentially have a short esophagus that leads to the stomach and empties into the intestine (Horn 1989; Helfman et al. 1997). However, there is some variety in the gut morphology among fishes. For example, elasmobranchs have a short, thick intestine with a large spiraling fold of tissue called the spiral valve, and some fishes only have reduced stomachs or lack them altogether (Horn 1989; Helfman et al. 1997). Teleosts generally have a longer intestine with numerous side pouches near the stomach called pyloric caecae (Helfman et al. 1997). Pyloric caecae are not found in all teleosts. For example, pyloric caecae are not present in channel catfish. Instead, the channel catfish has a J-shaped stomach that is connected to the intestinal tract by a pyloric sphincter (Grizzle and Rogers 1976).

11.3.3 Characterization of Fish Intestinal Microflora

Although the intestinal microbiota of many animal species has been examined thoroughly, this community has not been well characterized among fishes. To date, characterization of the microbiota associated with fishes has been restricted primarily to an examination of the microorganisms of potential concern to the food industry (i.e., those associated with food spoilage or human pathogenicity). For example, the areas studied include changes in flora during the storage of fish, effects based on catching or handling of the fish, relations between the environment and the fish microbiota, and the establishment of baseline data of microbial communities for monitoring changes in fish farms (Cahill 1990). Therefore, the composition and role of the intestinal microbial communities in fishes remain poorly understood. The cultivatable intestinal microbial community has been partially or fully characterized for only a fraction of fish species consisting mostly of freshwater fishes (Trust et al. 1979; Sakata et al. 1980, 1981; Campbell and Buswell 1983; Sugita et al. 1985, 2007; MacMillan and Santucci 1990; Cahill 1990; Luczkovich and Stellwag 1993; Spanggaard et al. 2000; Hagi et al. 2004; Huber et al. 2004; Burr et al. 2005, 2008a; Bairagi et al. 2002; Balcazar et al. 2008).

MacMillan and Santucci (1990) were interested in determining which bacteria are present in farm-raised channel catfish intestines and whether the types of bacteria present vary seasonally. They cultivated 858 isolates that comprised 20 genera and 26 species from the intestinal tract of channel catfish that were fed commercially prepared feed. In all, 31% of the isolates were facultative anaerobes, represented by pseudomonads or aeromonads. Campbell and Buswell (1983) partially characterized the microbial community in larval Dover sole and found differences

in the predominance of certain bacterial communities based on the diet of the fish. *Moraxella* spp. were most common in fish fed *Lumbricillus rivalis* (an oligochaete worm), representing 46% of the total isolates. *Vibrio* spp. and *Aeromonas* spp. were dominant in fish fed a pellet diet [composed of claws and waste material from decapod crustaceans, *Nephrops* sp. (40%), salmon starter food (57%), and pregelatinized starch (3%)] representing 52% of the isolates. Although these communities appear to be less diverse than termites and ruminants, the diversity of the microbial community may be limited because the farm-raised catfish and Dover sole consumed restricted diets. In addition, the authors did not intend to characterize the entire microbial community. In fact, the methods of isolation available at that time precluded isolation of the entire microbial community. In both the channel catfish and larval Dover sole, the cultivatable microbial community was dominated by facultative anaerobes. There appeared to be a relative absence of obligate anaerobes in the cultivatable microbial community.

11.3.4 Identification of Fish Intestinal Microflora

Even for species in which the microbiota has been fully characterized, the taxonomic affiliation of the bacteria must be seriously questioned because the isolates have not been thoroughly described and have generally been isolated in a manner inconsistent with the recovery of physiologically different bacteria. In fact, it is often highly probable that the normal intestinal microbiota were absent by the time the samples were processed for microbial isolation. Mitchell (1995) found that *Clostridium* 57-M-2′ spores were not maintained in the GI tract of pinfish after 16–24 h of capture from seagrass beds. This suggests that other bacteria may also disappear from the intestinal tract shortly after the fish leaves its natural habitat. Trust et al. (1979) were interested in examining the presence of obligate anaerobes in grass carp (*Ctenopharyngodon idella*), goldfish (*Carassius auratus*), and rainbow trout (*Oncorhynchus mykiss*). The fish were shipped to the laboratory and were in transit for approximately 36 h. All of the fish were subsequently grown in glass aquaria for 6 weeks prior to sampling. In all, 24 species of bacteria were isolated from grass carp, goldfish, and rainbow trout that were fed either pellet or weed diets. The three dominant microorganisms – *Aeromonas hydrophila*, *Pseudomonas* spp., *Yersinia enterocolitica* – represented 78% of the isolates cultured. The resident microbial community of these three fish species may have disappeared during transit or captivity.

This loss of the resident microbial community may also account for the absence of obligate anaerobes. Sugita et al. (1985) were interested in the interactions between the GI tract bacteria and the host fish. Carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), and tilapia (*Tilapia mossambica*) were purchased from a commercial supplier and reared for 1 month in plastic tanks. They cultured 11 species from the intestinal tract of carp, grass carp, and tilapia. All three species of fish were dominated by *Aeromonas hydrophila* and *Bacteroides* type A. The carp

examined in this study also had a significant number of *Citrobacter freundii*, *Pseudomonas* spp., and *Micrococcus* spp.; and tilapia had a significant number of *Bacteroides* type B. The resident microbial community of these fish may have disappeared from the intestinal tract during the time that they were reared in the laboratory. Most studies have implemented isolation methods that favor aerobic and facultative populations over anaerobic populations (Cahill 1990; Ringo 1993; Spanggaard et al. 2000; Huber et al. 2004). Therefore, conclusions based solely on data collected using aerobic methods have led some to believe that GI microbial communities of fish consist mainly of facultative anaerobic bacteria (Burr et al. 2005). This does not parallel results from other vertebrates. Thus, additional anaerobic studies and improved isolation methods are needed to understand the effects of the entire microbial community on the host (Burr et al. 2008a).

11.3.5   *Functionality of Fish Intestinal Microflora*

Along with the lack of knowledge about the composition of the community, little is known about the role of these microorganisms in the intestinal tract. It has been suggested that the intestinal microbial community may aid the fish host in dietary digestion processes. To receive any benefit from food consumed, fishes must produce the necessary enzymes to break down the many complex components of their diet. Herbivorous and omnivorous fishes and other vertebrates cannot produce, or may be deficient in, many hydrolytic enzymes such as cellulase, hemicellulase, chitinase, lignin peroxidase, or pectin esterase, which are enzymes required for hydrolysis of complex carbohydrates to a form that is nutritionally assimilable by the host (Luczkovich and Stellwag 1993; Sugita et al. 1997; Ramirez and Dixon 2003; Burr et al. 2005). One possible way for an organism to obtain deficient, but necessary, enzymes is to obtain them from the microbial communities that inhabit their intestinal tracts (Luczkovich and Stellwag 1993; Sugita et al. 1997; Bairagi et al. 2002; Ramirez and Dixon 2003; Burr et al. 2005).

Along with nutrients provided directly by the manufactured diet, aquaculture fishes may obtain an additional source of nutrients from their intestinal tract microbiota in a manner similar to that of ruminants. Until recently, herbivorous fishes were thought to lack the gut morphology necessary to support microbial fermentation. Hindgut caecae have been described and are thought to be a specialized fermentation chamber in some herbivorous fishes (Rimmer and Wiebe 1987). Burr et al. (2008a), Kihara and Sakata (2001, 2002), Smith et al. (1996), Titus and Ahearn (1991), and others have detected volatile fatty acids (VFAs) in red drum, rainbow trout and carp, largemouth bass, and tilapia, respectively, further indicating that fermentation is a common microbiological process in fish digestion. Furthermore, there is some indication that the fish GI tract is capable of sustaining microbial fermentation. Rimmer and Wiebe (1987) detected VFAs in the hindgut cecum of two kyphosids by steam distillation in a Markham still. The presence of VFAs suggested that fermentation had occurred, but the substrate of fermentation could not be

determined. They suggested that the fermentation appeared to have been facilitated by the microorganisms present in the intestine, although they did not characterize any microbiota. Burr et al. (2008a) measured the in vitro production of VFAs in cultured red drum and found the microbial community present in the intestinal contents to be mainly acetogenic. This contrasts with the VFA profiles of rainbow trout and carp intestinal contents studied by Kihara and Sakata (2001, 2002) wherein butyric and propionic were found in higher proportions. Differences were also found to be present between cool and warm temperate species (Kandel et al. 1994). *Cebidichthys violaceus*, a cool temperate species was solely acetogenic, whereas in *Medialuna californiensis*, a warm temperate species, only one-fifth of the VFAs present were acetate.

11.3.6 *Cellulolytic Bacteria in Fish Intestinal Tracts*

Given the economic advantage of supplementary catfish diets with higher fiber-containing components, there is an increasing interest in examining the potential for fiber digestibility in the intestinal tract. For example, cellulase activity, presumably due to cellulolytic bacteria, has been detected in the stomachs of the channel catfish, *Ictalurus punctatus* (Stickney and Shumway 1974). To determine the source of cellulase activity, Stickney and Shumway (1974) starved two groups of *I. punctatus* fingerlings for 5 days. One group served as controls, and the other group was administered streptomycin (200 mg/L) 24 h prior to determination of cellulose activity. The group exposed to the antibiotic yielded no detectable cellulase activity, whereas the other group continued to elicit cellulase activity. Indigenous cellulolytic bacteria that would be considered stable residents in the intestinal tract have been found in ruminants, such as cattle and sheep (Hungate 1966; Dehority and Varga 1991), in some insects such as termites (Odelson and Breznak 1983), and in some fishes (Smith 1992; Luczkovich and Stellwag 1993; Stellwag et al. 1995; Bairagi et al. 2002). Amylolytic, cellulolytic, lipolytic and proteolytic microflora have been isolated from the GI tract of catla, rohu, mrigal, silver carp, grass carp, common carp, tilapia, walking catfish, and murrel (Bairagi et al. 2002). Sugita et al. (1997) isolated amylase-producing intestinal microflora from ayu, carp, channel catfish, Japanese eel, and tilapia. Of the 206 strains isolated, 31.6% had the ability to produce 0.01–0.05 U amylase/ml. These digestive enzymes, produced by the intestinal microflora, may play an important role in fish host digestion processes.

It has also been shown that cellulolytic bacteria are present in the GI tract of pinfish, *Lagodon rhomboides* (Smith 1992; Luczkovich and Stellwag 1993; Stellwag et al. 1995). Pinfish are a logical species to harbor cellulolytic bacteria because they are found in considerable abundance in coastal seagrass meadows where they consume a vast amount of plant material. The consumption of live plants is an important stage of development in some species of fish. It has been demonstrated that pinfish undergo an ontogenetic change in diet from primarily carnivory to primarily herbivory (Carr and Adams 1973; Stoner and Livingston 1984; Luczkovich and

Stellwag 1993). A similar shift in diet has been observed among other fishes, such as *Diplodus sargus* and *Sarpa salpa* (Christensen 1977). Pinfish undergo changes in feeding behavior, dentition, and gut anatomy corresponding to an increase in the consumption of plant matter (Stoner and Livingston 1984; Luczkovich et al. 1995). Luczkovich and Stellwag (1993) determined that pinfish have cellulolytic microorganisms in their GI tract and were interested in how an ontogenetic change in diet might affect the resident intestinal microbial community of the pinfish. Two primary questions were proposed. First, there was the question as to whether a shift occurs in the intestinal microbial community as the fish changes from a predominantly carnivorous diet to a predominantly herbivorous diet. Second, if such a shift does occur, does the cellulase producer increase in relative abundance in the intestinal tract? Luczkovich and Stellwag (1993) found that changes in the proportion of plant matter consumed in the diet corresponded to changes in the proportion of cellulolytic microorganisms in the intestinal tract of pinfish. They concluded that pinfish have a microbial community with the enzymatic capacity to assist the host in acquiring nutrients from their diet that would otherwise be inaccessible. Similar microbial communities may be present in other species of fishes, particularly those that consume diets rich in plant matter or plant-derived detritus. If these communities exist in other fishes, it would be interesting to know whether cellulolytic microorganisms remain in the intestinal tract of a fish of aquacultural importance that consumes an artificial diet that is rich in plant material, such as the channel catfish, *Ictalurus punctatus.*

11.4   Culturing Fish Intestinal Microflora

Activity of enzymes associated with the intestinal microbial community is of little consequence if the numbers of organisms in the community are low. Given the size of the microbial community present, the numbers of microorganisms that have been cultured from the intestinal tract of fish are low. When compared to rumen bacterial levels of 10^{10}/g (Hungate et al. 1971), the average cultivatable bacterial levels in fish intestinal tract contents are considerably less, with results ranging from 10^2/g to 10^8/g (Trust et al. 1979; Sakata et al. 1980; Campbell and Buswell 1983; Sugita et al. 1985; Cahill 1990; Luczkovich and Stellwag 1993; Ringo et al. 1998; Ahilan et al. 2004; Hagi et al. 2004; Huber et al. 2004; Aubin et al. 2005; Bagheri et al. 2008). These levels can vary depending on seasonal changes in water temperature, the diversity of the microbial communities present in the fish intestinal tract (Hagi et al. 2004), and among individuals (Huber et al. 2004). However, the bacterial levels may have been greatly underestimated by some of the microbial techniques used, which may not select for species that are strict anaerobes and nutritionally fastidious. Likewise, the microbial composition of GI microflora has been difficult to estimate not only because of the changes that occur but the isolation methods used in some studies do not necessarily support the growth of the more oxygen-sensitive anaerobes (Mead 1997; Ricke and Pillai 1999; Ricke et al. 2004; Lungu et al. 2009).

Many of these studies were performed using bacteriological approaches under aerobic cultivation conditions, which would have been lethal to the obligate anaerobes. The few studies that have focused on anaerobes mostly used anaerobic jars, which are less efficient than anaerobic chambers (Sakata et al. 1980; Sugita et al. 1985; Seiderer et al. 1987; MacMillan and Santucci 1990; Summanen et al. 1999; Ramirez and Dixon 2003; Burr et al. 2008a) The environment in an anaerobic jar is made anaerobic by using hydrogen and a palladium catalyst to remove oxygen through the formation of water. However, each time the jars are opened oxygen enters and the gas pack must be changed. This addition of oxygen into the environment may be sufficient to kill the obligate anaerobes. The Anoxomat (Mart BV Microbiology Automation, Holland) and the Anaerobic Lap System (GR Instruments B.V., Wijk bij Duurstede, The Netherlands) are improved jar systems that use an automated evacuation-replacement technique to create an anaerobic environment (Summanen et al. 1999; Plugge 2005). Air is repeatedly removed from the sealed jar(s) by a vacuum and then filled with an anaerobic gas mixture. Like the traditional GasPak system, any remaining oxygen is removed using hydrogen and a palladium catalyst to remove oxygen through the formation of water. In addition to anaerobic jars, the Anaerobic Lap System can be connected to stoppered flasks and vials (Plugge 2005).

Anaerobic isolation methods and media developed for enumeration of the rumen anaerobic microflora (Hungate 1950) have been shown to be the best for studying predominantly anaerobic gut microflora in other animal species, resulting for example in the isolation of more than 200 strains of bacteria from the chicken ceca (Barnes and Impey 1972, 1980). Using a Bactron II anaerobe chamber (Anaerobe Systems, Morgan Hill, CA, USA) and prereduced anaerobically sterilized (PRAS) medium, Ramirez and Dixon (2003) isolated and cultivated 49 obligate anaerobic bacteria from the intestinal flora of *Astronotus ocellatus*, *Pterophyllum scalare*, and *Paralichthys lethostigma*. Unfortunately, knowledge of GI ecosystems is incomplete because even when strict anaerobic methodology is practiced only a portion of the total viable counts can be accounted for (Ricke and Pillai 1999; Mead 1997).

In contrast, an anaerobic chamber has an interchange compartment to allow materials to be placed inside the chamber without exposing the interior of the chamber to oxygen. The anaerobic atmosphere is maintained largely with a vacuum pump and nitrogen purges. The remaining oxygen is removed by a palladium catalyst and hydrogen. The oxygen reacts with the hydrogen to form water, which is then absorbed by a desiccant (Prescott et al. 1996). However, anaerobic chambers use considerable amounts of gas to maintain anaerobic conditions and are not capable of cultivating microorganisms with various environmental requirements (Plugge 2005).

The performance of the Anoxomat, the chamber, and the GasPak were compared by the growth of a variety of 54 obligate anaerobic bacteria strains and the recovery of anaerobic bacteria from 31 clinical specimens inoculated with known anaerobic bacteria strains (Summanen et al. 1999). Results indicated that the Anoxomat, the chamber, and the GasPak jars recovered 95%, 95%, and 93% of the 54 strains, respectively, and 93.5%, 94.4%, and 88.9% of the strains present in the specimens, respectively.

At this time, there is still no known medium that allows cultivation of all species of bacteria. The addition of reducing agents, such as thioglycolate, cysteine, palladium, or ascorbate may be used to eliminate any dissolved oxygen present in the medium (Shermer et al. 1998; Ricke and Schaefer 1990; Plugge 2005). Also, PRAS medium has been shown to improve isolation and cultivation of obligate anaerobes compared to aerobically prepared media (Chan et al. 1978; Ramirez and Dixon 2003). The combination of an appropriate medium and cultivation in a strictly anaerobic environment would allow growth of a large number of obligate and facultative anaerobes. Supplementation with rumen fluid supports optimal growth for some cecal isolates (Salanitro et al. 1974a, 1974b, 1978). This has been used an indication of chicken cecal bacteria possessing nutritional requirements similar to rumen organisms (Salanitro et al. 1974a, 1974b, 1978; Mead 1997). Based on approaches developed for rumen bacteria (Leedle and Hespell 1980), rumen fluid-based and non-rumen-fluid-based carbohydrate differential selective media for inoculation of plate medium in an anaerobic glove box have been successfully used for enumeration of chicken GI microflora (Shermer et al. 1998). The use of carbohydrate differential medium has allowed enumeration of total anaerobes on plates and distinguishes specific energy/nutrient bacterial populations that are responding to specific dietary changes such as lactose.

The intestinal microbiota that have been cultivated from fishes have not been thoroughly characterized. Therefore, the taxonomic affiliation of many isolates obtained from fishes remains in question. It is important to understand the composition of the intestinal microbial community to understand the influence that the intestinal microbiota have on host nutrition. Consequently, a more thorough characterization of the microbiota that evaluates phenotypic, chemotaxonomic, and genotypic data from both culture-dependent and culture-independent methodologies, a polyphasic approach (Schleifer 2009; Vandamme et al. 1996) is needed. Recent studies coupling culture-dependent and culture-independent methods give a new perspective of what is known and unknown about fish GI microbial communities in their entirety (Spanggaard et al. 2000; Huber et al. 2004; Pond et al. 2006; Kim et al. 2007). Utilizing conventional aerobic bacteria plate counts and molecular techniques, differential gradient gel electrophoresis (DGGE), 16S rDNA sequencing, and fluorescence in situ hybridization (FISH) analyses, Huber et al. (2004) identified the dominant culturable and nonculturable microbiota present in the intestines of rainbow trout. Overall, the composition of the microbial communities varied from fish to fish. Sample culturability rates were found to be 50–90%, and Proteobacteria were the dominant GI residents. Samples with unculturable bacteria (aerobic bacteria plate counts were <2% of the direct microscopic counts) were subjected to additional molecular testing for identification. They were shown to have similarity values of 98%, 95%, and 89% with *Carnobacteria*, *Clostridia*, and *Anaerofilum pentosovorans*, respectively. Conventional microbiological and

molecular techniques are essential for all bacteria to be represented when defining relationships within a microbiome (Spanggaard et al. 2000; Huber et al. 2004; Pond et al. 2006; Kim et al. 2007).

11.5 Manipulation of the Intestinal Tract Microflora

11.5.1 General Concepts

An increased understanding of the intestinal microflora in commercial fish, such as catfish, has potential for manipulation or alteration to enhance disease resistance and growth performance. Changing or replacing intestinal microflora to benefit the host can be done by dietary manipulation to select for certain subgroups of intestinal bacteria, resulting in a shift of the total population (Gatesoupe 2002; Aubin et al. 2005; Balcazar et al. 2008; Merrifield et al. 2009). Dietary manipulations can be carried out using cultures that are referred to as probiotics or competitive exclusion cultures (Merrifield et al. 2009). A further development of this concept is to add dietary amendments or prebiotics that are not digestible but can be utilized by microorganisms such as lactic acid bacteria. They are considered particularly beneficial either directly by the production of metabolic products that enhance the host in some way or indirectly by serving as a barrier to organisms considered harmful to the host (Burr et al. 2005, 2008a, 2008b; Merrifield et al. 2010; Ringo et al. 2010).

11.5.2 Probiotics

11.5.2.1 General Concepts

A probiotic involves the supplementation of actual viable microorganisms that potentially can become established in the GI tract and provide benefits to the host. Merrifield et al. (2010) outlined criteria for an ideal probiotic. An ideal probiotic is able to colonize the intestinal epithelial surface, is resistant to bile salts and low pH, and should have antagonistic characteristics toward common host pathogens. In addition, it was stressed that it must be free of plasmid-encoded antibiotic resistance genes and be nonpathogenic to aquatic animals and human consumers in addition to the host species. Reported beneficial effects in cattle, pigs, poultry, and fish include improved general health, more efficient food utilization, faster growth rate, and increased milk and egg production (Fuller 1992, 1997; Berg 1998; Gatesoupe 2002; Aubin et al. 2005; Balcazar et al. 2008; Merrifield et al. 2009). Obviously, such benefits have led to tremendous commercial growth in marketing such products, but considerable controversy exists as to how direct the influence of adding biological additives is in eliciting these benefits.

11.5.2.2   Probiotics in Aquaculture

Recent aquaculture feeding trials with probiotics have suggested that competitive exclusion and enhanced physiologicial and immune responses contribute to observed improvements in aquaculture health (Gatesoupe 2002; Aubin et al. 2005; Carnevali et al. 2006; Balcazar et al. 2008; Merrifield et al. 2009; Van Hai and Fotedar 2009; Castex et al. 2010). Lactic acid bacteria are thought to promote growth in European sea bass juveniles (*Dicentrarchus labrax*) by reducing cortisol and promoting transcription of genes responsible for growth (Carnevali et al. 2006). Antioxidant status and oxidative stress were monitored in blue shrimp (*Litopenaeus stylirostris*) fed a diet supplemented with *Pediococcus acidilacti* MA18/5M for 1 month and then exposed to pathogenic *Vibrio nigripulchritudo* (Castex et al. 2010)*.* Compared to the infected and uninfected control groups, infected shrimps fed the probiotic diet maintained antioxidant status and oxidative stress levels similar to the uninfected control group. Gatesoupe (2002) used *P. acidilactici* to supplement an *Artemia nauplii* diet and found improved growth in an experiment involving larval Pollack compared to *Artemia nauplii* alone.

Effects differ among species and the type of probiotic used, as well as the dosage and length of time it is administered (Gatesoupe 2002; Aubin et al. 2005; Carnevali et al. 2006; Sugita et al. 2007; Balcazar et al. 2008; Merrifield et al. 2009; Van Hai and Fotedar 2009; Castex et al. 2010). The extent of prevention from vertebral column compression syndrome (VCCS), a common spinal deformity characterized by width reduction and fusion of the vertebrae, significantly increased in rainbow trout (*Oncorhynchus mykiss* Walbaum) when they were fed a basal diet supplemented with probiotics (Aubin et al. 2005). A control diet and five experimental diets, each combined with a different supplement and administered for different amounts of time were compared after an experimental period of 5 months. The control group was fed Ecoweaner followed by Ecostart (Biomar S.A., Nersac, France), depending on the size of the fingerlings, for the entire experimental period. A second group was fed the control diet supplemented with the antibiotic florfenicol (final concentration 18 mg kg^{-1}) (Nuflor, Schering-Plough, Union, NJ, USA) for the first 10 days and the control diet alone for the remainder of the experimental period. Two of groups were fed the control diet supplemented with one of two probiotics, *Pediococcus acidilactici* MA18/ 5M or *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 for 20 days and the control diet for the remainder of the experimental period. The remaining two groups were also fed the control diet supplemented with one of the two probiotics for the entire 5-month period. *P. acidilactici* and *S. cerevisiae* were administered in concentrations of $1.5 \pm 0.4 \times 10^6$ and $4.0 \pm 0.1 \times 10^6$, respectively. No significant differences were found in the occurrence of VCCS between the control group (13%) and the groups administered the probiotic *S. cerevisiae* (9–10%). Fish fed the diets supplemented with either the antibiotic for 10 days or the probiotic *Pediococcus acidilactic* for the entire 5 months were significantly different from the control group (3% and 4%, respectively). However, in the groups fed the probiotic *P. acidilactic* for only 10 days there was no significant effect on the rate of VCCS (12%) (Aubin et al. 2005). Similar results occurred in

Table 11.1 Probiotics and prebiotics evaluated in catfish species

Species	Probiotic/prebiotic	Effects	Reference
African catfish (*Clarias gariepinus*)	*Lactobacillus acidophilus*	↑ Growth, survival	Al-Dohail et al. (2009)
Juvenile channel catfish (*Ictalurus punctatus*)	*Enterococcus* (Biomate SF-20)	→ Growth and disease resistance	Shelby et al. (2007)
	Bacillus subtilis and *B. licheniformis* (Bioplus 2B)	→ Growth and disease resistance	
	Pediococcus (Bactocell PA10 MD)	→ Growth and disease resistance	
	Lactobacillus (LA-51)	→ Growth and disease resistance	
	11 *Bacillus* spp. (Clear-Flo 1002)	→ Growth and disease resistance	
	14-g + 8-g species (Clear-Flo 1005)	→ Growth and disease resistance	
	6-g + 10-g species (Clear-Flo 1006)	→ Growth and disease resistance	
African catfish (*Clarias gariepinus*)	AXOS	↑ Acetate, propionate, and total SCFAs	Rurangwa et al. (2008)
Channel catfish (*Ictalurus punctatus*)	Bio-Mos	→ Weight gain, feed conversion, survival rates	Welker et al. (2007)
Channel catfish (*Ictalurus punctatus*)	Bio-Mos	↑Survival; → weight gain, feed conversion rates	Peterson et al. 2010)
→ No effect	↑ Increased	↓ Decreased	

SCFAs short-chain fatty acids, *ASOX* arabinoxylooligosaccharides

European sea bass juveniles (*Dicentrarchus labrax*) given feed supplemented with lactic acid bacteria (Carnevali et al. 2006). Fish treated for 59 days had a higher body weight (81%) compared to the fish treated for 25 days (28%) with respect to the fish given feed without probiotics.

11.5.2.3 Probiotics in Catfish

Limited information is available on probiotic effects on catfish, and the results that have been reported vary (Table 11.1). *Lactococcus lactis*, isolated from Amur catfish (*Silurus asotus*), has been shown to elicit beneficial effects by producing hydrogen peroxide (Sugita et al. 2007). The hydrogen peroxide inhibited the growth of opportunistic pathogens *Aeromonas hydrophila* and *A. caviae* (Sugita et al. 2007). In more recent work, a 12-week feeding trial was conducted using *Lactobacillus acidophilus* as a probiotic for African catfish (*Clarias gariepinus*) (Al-Dohail et al. 2009). Fingerlings were fed two diets: one group with and one group without *L. acidophilus*-supplemented feed. The mean final weight increased

from 155.43 ± 8.23 g for the control group to 182.04 ± 3.75 g for the experimental group. Total immunoglobulin for the control and experimental groups increased from 7.40 ± 0.33 (mg mL^{-1}) to 9.50 ± 0.14 (mg mL^{-1}), respectively. Given that significant increases were observed in growth performance, survival, and hematology/ immunology parameters, Al-Dohail et al. (2009) concluded that *Lactobacillus acidophilus* is potentially an acceptable probiotic for the African catfish.

Commercially available probiotics were combined with a commercial diet individually and in combination in an effort to assess the effects on growth and disease resistance in juvenile channel catfish (*Ictalurus punctatus*) (Shelby et al. 2007). Probiotics included *Enterococcus* (Biomate SF-20), *Bacillus* (Bioplus 2B), *Pediococcus* (Bactocell PA10 MD), and *Lactobacillus* (LA-51) species and were administered following the manufacturer's recommendations during 5- and 8-week feeding trials. Following the feeding trials, fish were challenged with *Edwardsiella ictaluri* and monitored for an additional 14 days. No significant increases in growth or disease resistance were found (Shelby et al. 2007).

Shelby et al. (2007) stressed that despite recent successes in probiotic studies, probiotics need to be customized to suit individual species and age groups for positive effects to be achieved by the aquaculturist, the end-user, on a large scale. Further research is needed to understand the mechanisms of probiotics for individual species. Most mechanisms that have been suggested to explain apparent successes in animals are too simplistic ecologically when the complexities of the GI tract are considered. Thus, the problem remains that even when such biological additives appear to work the mechanism(s) are still not understood.

11.5.3 Prebiotics

11.5.3.1 General Concepts

Prebiotics involve some sort of selective additive that is not utilizable by the host animal but can be selectively used and therefore is stimulatory to a portion of the GI population which is then presumed beneficial to the host (Orban et al. 1997; Berg 1998; Ringo et al. 2010). Prebiotics that support positive bacteria populations such as *Lactobacillus* are comprised of carbohydrates, each classified by the number of monosaccharide units (Niness 1999; Ringo et al.2010). When they are used in combination with probiotics, they are termed synbiotics (Gibson and Roberfroid 1995). The practice of supplementing prebiotic additives to domestic animals' diets during production is becoming widespread. There is precedence for improving dietary protein digestibility by prebiotic addition as well. In chickens, a prebiotic-enzyme preparation added to broiler chicken diets containing up to 20% poultry by-product meal increased protein efficiency and feed conversion efficiency (Kirkpinar et al. 2004). In addition to stimulating the growth of select populations of intestinal bacteria, there is evidence to suggest that prebiotics can improve the availability of important nutrients such as calcium and iron (Teitelbaum and Walker 2002).

11.5.3.2 Prebiotics in Aquaculture

Only limited studies with prebiotics have been conducted in fish (Table 11.1). The effects of fructooligosaccharide (FOS), galactooligosaccharide (GOS), mannanoligosaccharides (MOS), xyloooligosaccharides (XOS), arabinoxylooligosaccharides (AXOS), isomaltooligosaccharides (IMO), inulin (a water-soluble dietary fiber), and GroBiotic AE/GroBiotic-A (a mixture of partially autolyzed brewer's yeast, dairy ingredient components, and dried fermentation products) on GI populations in aquaculture have been evaluated (Li and Gatlin 2004; Ringo et al. 2006; Welker et al. 2007; Burr et al. 2008a, 2008b; Rurangwa et al. 2008; Li et al. 2009; Xu et al. 2009; Peterson et al. 2010). Improvements observed in host health include increased acetate, propionate, and total short-chain fatty acids (SCFAs) in both African catfish (*Clarias gariepinus*) and Siberian sturgeon given a basal diet supplemented with AXOS for 10 weeks (Rurangwa et al. 2008; Ringo et al. 2010). The effects of three basal diets, one supplemented with 50 kg^{-1} XOS, the second supplemented with 100 kg^{-1} XOS, and the third supplemented with 200 kg^{-1} XOS were compared to the basal diet alone administered to Crucian carp (*Carassius auratus gibelio*) for 45 days. The diet supplemented with 100 kg^{-1} XOS, significantly increased growth and enzymatic activity compared to the basal diet alone (Xu et al. 2009).

The effects of Grobiotic AE were investigated with hybrid striped bass (Li and Gatlin 2004). Over the course of 21 weeks, bass exposed to the fish pathogen *Mycobacterium marinum* were fed a basal diet supplemented with 2% Grobiotic AE and 1% or 2% brewer's yeast. Li and Gatlin (2004) reported that a diet containing Grobiotic AE and brewer's yeast yielded a significantly higher feed efficiency. Those fish fed the prebiotic supplement also had significantly lower mortality (20%) when challenged with the bacterial pathogen, *Streptococcus iniae,* compared to the fish fed the basal diet alone (approximately 28%). Prebiotics are a potential replacement of antibiotics, and as Li and Gatlin (2004) pointed out, they also offer a practical approach to make poultry by-product meal more attractive as a possible protein substitute for fish meal in aquatic feeds. This is important because aquaculture's consumption and cost of fishmeal and fish oil has substantially increased (Naylor et al. 2009). However, to optimize the type of prebiotic most suited for feeding with poultry by-product requires initial in vitro screening after incubation with hybrid striped bass intestinal microflora to determine protein digestibility and nutrient availability effects (Li and Gatlin 2004).

GroBiotic-A, MOS, and GOS also exhibited positive effects in nutrient digestibility for red drum (*Sciaenops ocellatus*) (Burr et al. 2008b); and when added individually to a basal diet consisting of soybean meal (50%) and mehaden fish meal (50%), the apparent digestibility coefficient (ADC) for energy, protein, and organic matter significantly increased. In a separate study conducted in an anaerobic chamber, intestinal contents were removed from three red drum (*Sciaenops ocellatus*) previously fed a basal diet (Burr et al. 2008a). The intestinal contents were used as inoculum in one of four liquid media, three of which contained a basal diet supplemented with 2% GroBiotic-A, 2% brewer's yeast, or 2% FOS; the fourth contained the basal diet alone as a control. Aliquots (1 mL) of each sample were used for VFA and DGGE analysis after incubation for 0, 24, and 48 h at 25°C. After 24 h, samples

supplemented with GroBiotic-A significantly increased acetate (88.73 ± 86.9 μmol/mL) compared to the brewer's yeast (42.88 ± 17.0 μmol/mL), FOS (26.99 ± 12.5 μmol/mL), and basal diet (16.18 ± 12.9 μmol/mL) alone. After 48 h, GroBiotic-A exhibited the highest acetate (67.85 ± 19.2) and total VFA production (78.13 ± 16.1) but was not significantly different from the other samples. DGGE analysis revealed significant differences in the composition of the microbial communities between the 24-h and 48-h samples. The bacterial species present in the 24-h samples were determined to be highly related or identical, whereas the bacterial species in the 48-h samples were much more diverse. Significant differences were also observed in the bacterial species stimulated by GroBiotic-A and the bacterial species stimulated by brewer's yeast.

11.5.4   *Prebiotics in Catfish*

Feeds supplemented with MOS have had various effects on a wide range of aquatic species. Red drum (Burr et al. 2008b), rainbow trout, hybrid tilapia, and tiger shrimp have shown positive improvements in response to MOS (Staykov et al. 2007; Genc et al. 2007a, 2007b). The effects of Bio-Mos-supplemented catfish diets on the growth and survival of channel catfish, when challenged with *Edwardsiella ictaluri*, were evaluated by Welker et al. (2007) and Peterson et al. (2010). No significant increases in weight gain or feed conversion rates were observed. In the 6-week feeding trials followed by a 21-day challenge with *Edwardsiella ictaluri* by Peterson et al. (2010), survival rates differed between the diet with 36% crude protein supplemented with Bio-Mos (2 g/kg of control diet) and diet prepared by extrusion technology with 32% crude protein supplemented with Bio-Mos (2 g/kg of control diet). The 36% crude protein diet supplemented with Bio-Mos improved survival by 42%, whereas the 32% crude protein diet showed no significant effect on survival when challenged with *Edwardsiella ictaluri.*

   Welker et al. (2007) fed a 32% crude protein control diet supplemented with Bio-Mos (2 g/kg of control diet) for 4 weeks. The fish were subsequently fed the 32% crude protein control diet alone for 2 weeks and then challenged with *Edwardsiella ictaluri.* Survival rates (5.0–17.5%) did not significantly improve, and it was suggested by Peterson et al. (2010) that the benefits of the prebiotic may have decreased during the 2 weeks prior to the challenge with *Edwardsiella ictaluri* when fed the control diet alone. Welker et al. (2007) measured immune parameters (plasma lysozyme, bactericidal, spontaneous hemolytic complement activities) at 4 and 6 weeks; and whereas values were significantly different between the 4- and 6-week measurements, values did not vary between the control diet and the diet supplemented with Bio-Mos. Peterson et al. (2010) also monitored lysozyme activity before and after the challenge and found that lysozyme activity was similar between the diets. Such findings highlight the need to understand better the inner workings between a host's GI tract and its inhabitants to exploit successfully the use of prebiotics in channel catfish and for aquaculture in general.

11.6 Conclusions and Future Research

The U.S. catfish industry has a large economic impact, generating billions of dollars annually. Catfish products worth US$445 billion were sold by growers to processors in 2007 (Haley 2008). Despite this figure, the total was down 8% from the previous year; and with recent rises in energy and feed costs, catfish industry profits, farm acreage, and employment have decreased during the last 7 years (Harvey 2006; Haley 2008; Naylor et al. 2009). A practical reason for looking at the microbial community of channel catfish is the potential for lowering feed costs. If a stable intestinal microbial community exists, it might be possible to manipulate the microorganisms in the community in such a way as to allow the channel catfish to consume a diet high in formulated plant matter, which is more inexpensive and a readily available food source such as crop residues.

Fish GI microorganisms have received periodic interest with the isolation of individual organisms usually accompanied by identification and characterization studies. Relating these findings back to the ecology of the fish GI tract and the potential impact on the nutrition and health of the fish have been the focus of most of these studies. The repeated successes with the application of prebiotics and competitive exclusion or probiotic cultures ensures continued commercialization and widespread use individually and in combination (Ricke and Pillai 1999; Gatesoupe 2002; Aubin et al. 2005; Burr et al. 2005; Merrifield et al. 2009). Recently, the European Standing Committee on the Food Chain and Animal Health authorized the use of probiotics in aquaculture (Lallemand Animal Nutrition 2009). However, additional feeding trials are needed to identify specific beneficial strains for individual species, as are investigations of how combinations of prebiotics and probiotics (synbiotics) can maintain both the productivity of beneficial bacteria strains and their presence in the GI tract. What is really needed is an understanding of the key processes of bacteria, particularly anaerobes, in the GI system that lead to the subsequent fermentation characteristics and ecological balance exhibited by beneficial microflora. This requires a much more complete picture of the GI microbial ecology and should include strict anaerobes such as methanogens, which have historically been overlooked when GI microflora have been characterized.

References

Ahilan, B., G. Shine, and R. Santhanam. 2004. Influence of probiotics on the growth and gut microbial load of juvenile goldfish (*Carassius auratus*). *Asian Fisheries Science* 17: 271–278.
Al-Dohail, M.A., R. Hashim, and M. Aliyu-Paiko. 2009. Effects of the probiotic, *Lactobacillus acidophilus*, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquaculture Research* 40: 1642–1652.

Andrews, J.W., and R.R. Stickney. 1972. Interactions of feeding rates and environmental temperature on growth, food conversion, and body composition of channel catfish. *Transactions of the American Fisheries Society* 101: 94–99.

Atlas, R.M., and R. Bartha. 1993. *Microbial ecology: Fundamentals and applications*, vol. 2, 112–115. Redwood City: The Benjamin/Cummings Publishing Company, Inc.

Aubin, J., F.J. Gatesoupe, L. Labbe, and L. Lebrun. 2005. Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture Research* 36: 758–767.

Bagheri, T., S. Hedayati, V. Yavari, M. Alizade, and A. Farzanfar. 2008. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. *Turkish Journal of Fisheries and Aquatic Sciences* 8: 43–48.

Bailey, R.M., and H.M. Harrison. 1948. Food habits of the Southern Channel Catfish (*Ictalurus Lacustris punctatus*) in the Des Moines River, Iowa. *Transactions of the American Fisheries Society* 75: 110–138.

Bairagi, A., K.S. Ghosh, S.K. Sen, and A.K. Ray. 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. *Aquaculture International* 10: 109–121.

Balcazar, J.L., D. Vendrell, I. de Blas, I. Ruiz-Zarzuela, J.L. Muzquiz, and O. Girones. 2008. Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. *Aquaculture* 278: 188–191.

Baldwin, R.L., and M.J. Allison. 1983. Rumen metabolism. *Journal of Animal Science* 57: 461–477.

Barnes, E.M., and C.S. Impey. 1972. Some properties of the nonsporing anaerobes from poultry ceca. *Journal of Applied Bacteriology* 35: 241–251.

Barnes, E.M., C.S. Impey, and D.M. Cooper. 1980. Manipulation of the crop and intestinal flora of the newly hatched chick. *American Journal of Clinical Nutrition* 33: 2426–2433.

Berg, R.D. 1998. Probiotics, prebiotics or 'conbiotics'? *Trends in Microbiology* 6: 89–92.

Brauman, A., J. Dore, P. Eggleton, D. Bignell, J.A. Breznak, and M.D. Kane. 2001. Molecular phylogenetic profiling of prokaryotic communities in guts of termites with different feeding habits. *FEMS Microbiology Ecology* 35: 27–36.

Breznak, J.A. 1984. Biochemical aspects of symbiosis between termites and their intestinal microbiota. In *Invertebrate–microbial interactions*, ed. J.M. Anderson, A.D.M. Rayner, and D.W.H. Walton. Cambridge: Cambridge University Press.

Burch, T.A. 1970. *Estimated costs and returns of channel catfish production, Beaufort-Jasper Area, South Carolina. Misc. Ext. Circular*. Clemson University and U.S. Dept. of Agriculture Extension Service.

Burr, G., S. Ricke, and D. Gatlin. 2005. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish. *Journal of the World Aquaculture Society* 36: 425–436.

Burr, G., M. Hume, S. Ricke, D. Nisbet, and D. Gatlin. 2008a. A preliminary *in vitro* assessment of GroBiotic®-A, brewer's yeast and fructooligosaccharide as prebiotics for the red drum *Sciaenops ocellatus*. *Journal of Environmental Science and Health Part B* 43: 253–260.

Burr, G., M. Hume, W.H. Neill, and D.M. Gatlin. 2008b. Effects of prebiotics on nutrient digestibility of a soybean-meal-based diet by red drum *Sciaenops ocellatus* (Linnaeus). *Aquaculture Research* 39: 1680–1686.

Cahill, M.M. 1990. Bacterial flora of fishes: A review. *Microbial Ecology* 19: 21–41.

Campbell, A.C., and J.A. Buswell. 1983. The intestinal microflora of farmed Dover sole (*Solea solea*) at different stages of fish development. *Journal of Applied Bacteriology* 55: 215–223.

Carnevali, O., L. de Vivo, R. Sulpizio, G. Gioacchini, I. Olivotto, S. Silvi, and A. Cresi. 2006. Growth improvement by probiotic in European sea bass juveniles (Dicent*rarchus labrx* L.), with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquaculture* 258: 430–438.

Carr, W.E.S., and C.A. Adams. 1973. Food habits of juvenile marine fishes occupying seagrass beds in the estuarmne zone near Crystal River, Florida. *Transactions of the American Fisheries Society* 102: 511–540.

Carter, R.R., and K.O. Allen. 1976. Effects of flow rate and aeration on survival and growth of channel catfish in tanks. *Progressive Fish-Culturist* 38: 204–206.

Castex, M., P. Lemaire, N. Wabete, and L. Chim. 2010. Effect of probiotic *Pediococcus acidilactici* on antioxidant defenses and oxidative stress of *Litopenaeus stylirostris* under *Vibrio nigripulchritudo* challenge. *Fish Shell Immunology* 28: 622–631.

Chan, E.C., J. Devries, and R.F. Harvey. 1978. Preparation of prereduced anaerobically sterilized media and their use in cultivation of anaerobic bacteria. *Journal of Clinical Microbiology* 8: 123–126.

Christensen, M.S. 1977. Trophic relationships in juveniles of three species of sparid fishes in the South African marine littoral. *Fishery Bulletin U.S.* 76: 389–401.

Collado, M.C., and Y. Sanz. 2007. Quantification of mucosa-adhered microbiota of lambs and calves by the use of culture methods and fluorescent *in situ* hybridization coupled with flow cytometry techniques. *Veterinary Microbiology* 121: 299–306.

Dehority, B.A., and G.A. Varga. 1991. Bacterial and fungal numbers in ruminal and caecal contents of the Blue Duiker (*Cephalophus nionticola*). *Applied and Environmental Microbiology* 57: 469–472.

Dunning, R. 1995. *Aquaculture in North Carolina: Catfish*. Raleigh: North Carolina Dept. of Agric. Div. of Aqua. and Nat. Res.

Eddy, S., and J.C. Underhill. 1974. *Northern fishes*. Minneapolis: University of Minnesota Press.

Federal Coordinating Council on Science, Engineering and Technology. 1983. *The joint subcommittee on aquaculture*, Vol. 2. Washington D.C.

Forbes, S.A. 1888. Studies of the food of fresh-water fishes. *Bulletin of the Illinois State Laboratory of Natural History* 2: 433–473.

Fuller, R. 1992. History and development of probiotics. In *Probiotics the scientific basis*, ed. R. Fuller. London: Chapman & Hall.

Fuller, R. 1997. Introduction. In *Applications and practical aspects*, vol. 2, ed. R. Fuller. New York: Chapman & Hall.

Gatesoupe, F.J. 2002. Probiotic and formaldehyde treatments of *Artemia nauplii* as food for larval Pollack, *Pollachius pollachius*. *Aquaculture* 21: 347–360.

Genc, M.A., E. Yilmaz, E. Genc, and M. Aktas. 2007a. Effects of dietary mannan oligosaccharides (MOS) on growth, body composition, and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus* x *O aureus*). *Israeli Journal of Aquaculture-Bamidgeh* 59: 10–16.

Genc, M.A., M. Aktas, E. Genc, and E. Yilmaz. 2007b. Effects of dietary mannan oligosaccharides on growth, body composition and hepatopancreas histology of *Penaeus semisulcatus* (de Haan 1844). *Aquaculture Nutrition* 13: 156–161.

Gibson, G.R., and M.B. Roberfroid. 1995. Dietary modulation of the human colonic microbiotia: Introducing the concept of prebiotics. *Journal of Nutrition* 125: 1401–1412.

Goddard, S. 1996. *Feed management in intensive aquaculture*, vol. 87, 15–18. New York: Chapman & Hill.

Green, C.C., and A.M. Kelly. 2009. Effects of the estrogen mimic genistein as a dietary component on sex differentiation and ethoxyresorufin-O-deethylase (EROD) activity in channel catfish (*Ictalurus punctatus*). *Fish Physiology and Biochemistry* 35: 377–384.

Grizzle, J.M., and W.A. Rogers. 1976. *Anatomy and histology of the channel catfish, Ictalurus punctatus*, 19–28. Auburn: Auburn Printing.

Hagi, T., D. Tanaka, Y. Iwamura, and T. Hoshino. 2004. Diversity and seasonal changes in lactic acid bacteria in the intestinal tract of cultured freshwater fish. *Aquaculture* 234: 335–346.

Haley, M.M. 2008. *Livestock, Dairy and Poultry Outlook. Economic Research Service USDA*. http://www.ers.usda.gov/publications/ldp. Accessed July 15, 2010.

Harvey, D.J. 2006. *Aquaculture Outlook. Economic Research Service USDA*. http://usda.mannlib.cornell.edu/usda/ers/LDP-AQS//2000s/2006/LDP-AQS-10-05-2006.pdf. Accessed July 15, 2010.

Hastings, W.H., and L.M. Dickie. 1972. Feed formulation and evaluation. In *Fish nutrition*, ed. J.E. Halver. New York: Academic.

Helfman, G.S., B.B. Collette, and D.E. Facey. 1997. *The diversity of fishes*, vol. 3, 66. Maiden: Blackwell.

Hepher, B. 1988. *Nutrition of pond fishes*, 292–294. Cambridge: Cambridge University Press.

Horn, M.H. 1989. Biology of marine herbivorous fishes. *Oceanography and Marine Bioliogy Annual Review* 27: 167–272.

Huber, I., B. Spanggaard, K.F. Appel, L. Rossen, T. Nielsen, and L. Gram. 2004. Phylogenetic analysis and *in situ* identification of the intestinal microbial community of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Applied Microbiology* 96: 117–132.

Hungate, R.E. 1950. The anaerobic mesophilic cellulolytic bacteria. *Bacteriological Reviews* 14: 1–49.

Hungate, R.E. 1966. *The rumen and its microbes*. New York: Academic.

Hungate, R.E., J. Reich, and R. Prins. 1971. Parameters of rumen fermentation in a continuously fed sheep: Evidence of a microbial rumination pool. *Applied Microbiology* 22: 1104–1113.

Jordan, D.S., and B.W. Evermann. 1908. *American food and game fishes*, 21–22. New York: Doubleday, Page and Company.

Kandel, J.S., M.H. Horn, and W. Vanantwerp. 1994. Volatile fatty-acids in the hindgut of herbivorous fishes from temperate and tropical marine waters. *Journal of Fish Biology* 45: 527–529.

Kihara, M., and T. Sakata. 2001. Influences of incubation temperature and various saccharides on the production of organic acids and gases by gut microbes of rainbow trout *Oncorhynchus mykiss* in a micro-scale batch culture. *Journal of Comparative Physiology B* 171: 441–447.

Kihara, M., and T. Sakata. 2002. Production of short-chain fatty acids and gas from various oligo-saccharides by gut microbes of carp (*Cyprinus carpio L.*) in micro-scale batch culture. *Comparative Biochemistry and Physiology* A 132: 333–340.

Kim, D., J. Brunt, and B. Austin. 2007. Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Microbiology* 102: 1654–1664.

Kirkpinar, F., Z. Açigöz, M. Bozkurt, and V. Ayhan. 2004. Effects of inclusion of poultry by-product meal and enzyme-prebiotic supplementation in grower diets on performance and feed digestibility of broilers. *British Poultry Science* 45: 273–279.

Lallemand Animal Nutrition. 2009. *BACTOCELL®: The First Probiotic Authorized for use in Aquaculture in the European Union*. http://www.lallemand.com/Home/eng/PDFs/LAN09-PR09-Bactocell_aqua_EN.pdf.

Leedle, J.A.Z., and R.B. Hespell. 1980. Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate-utilizing subgroups in rumen bacterial-populations. *Applied and Environmental Microbiology* 39: 709–719.

Li, P., and D.M. Gatlin III. 2004. Dietary brewers yeast and the prebiotic GrobioticTMAE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* x *M. saxatilis*.) to *Streptococcus iniae* infection. *Aquaculture* 231: 445–456.

Li, J., B. Tan, and K. Mai. 2009. Dietary probiotic *Bacillus* OJ and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (*Litopenaeus vannamei*). *Aquaculture* 291: 35–40.

Lim, C., M. Yildirim-Askoy, and P.H. Klesius. 2009. Growth response and resistance to *Edwardsiella ictaluri*, fed diets containing distiller's dried grains and solubles. *Journal of the World Aquaculture Society* 40: 182–193.

Lovell, T. 1989. *Nutrition and feeding of fish*, 111–114. New York: Van Norstrand Reinhold.

Luczkovich, J.J., and E.J. Stellwag. 1993. Isolation of cellulolytic microbes from the intestinal tract of the pinfish, *Lagodon rhomboides*: Size-related changes in diet and microbial abundance. *Marine Biology* 116: 381–388.

Luczkovich, J.J., S.F. Norton, and R.G. Gilmore. 1995. The influence of oral anatomy on prey selection during ontogeny of two percoid fishes, *Lagodon rhomboides* and *Centropomus undecirnalis*. *Environmental Biology of Fishes* 44: 79–95.

Lungu, B., S.C. Ricke, and M.G. Johnson. 2009. Growth survival, proliferation and pathogenesis of *Listeria monocytogenes* under low oxygen or anaerobic conditions: A review. *Anaerobe* 15: 7–17.

MacMillan, J.R., and T. Santucci. 1990. Seasonal trends in intestinal bacterial flora of farm-raised channel catfish. *Journal of Aquatic Animal Health* 2: 217–222.

Malinen, E., T. Rinttila, K. Kajandar, J. Matto, and A. Kassinen. 2005. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with realtime PCR. *American Journal of Gastroenterology* 100: 373–382.

McNab, J.M. 1973. The avian caeca: A review. *Worlds Poultry Science Journal* 29: 251–263.

Mead, G.C. 1997. Bacteria in the gastrointestinal tract of birds. In *Gastrointestinal microbiology*, vol. 2, ed. R.I. Mackie, B.A. White, and R.E. Isaacson, 216–240. London: Chapman & Hall.

Merrifield, D.L., G. Bradley, G.M. Harper, R.T.M. Baker, C.B. Munn, and S.J. Davies. 2009. Assessment of the effects of vegetative and lyophilized *Pediococcus acidilactici* on growth, feed utilization, intestinal colonization and health parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aqua Nutr*. DOI: 10.1111/j.1365-2095.2009.00712.x

Merrifield, D.L., A. Dimitroglou, A. Foey, S.J. Davies, R.T.M. Baker, J. Bogwald, M. Castex, and E. Ringo. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* 302: 1–18.

Mississippi Agricultural and Forestry Experiment Station, Mississippi State University Extension Service. 2010. *Commercial Catfish Production*. http://msucares.com/aquaculture/catfish/index. html. Accessed Jan 5, 2010. Washington D.C., The Joint Subcommittee on Aquaculture.

Mitchell, T.G. 1995. An investigation of the ecological relationship between a carboxymethylcellulase-producing bacterium and pinfish, *Lagodon rhomboides*. Masters thesis. Greenville 53: East Carolina University.

Moore, W.E.C., and L.V. Holdeman. 1974. Human fecal flora: The normal flora of 20 Japanese-Hawaiians. *Applied Microbiology* 27: 961–979.

Moyle, P.B., and J.J. Cech. 1988. *Fishes: An introduction to ichthyology*, 2nd ed. Englewood Cliffs: Prentice Hall.

Naylor, R., R. Hardy, D. Bureau, A. Chlu, M. Elliott, A. Farrell, I. Forster, D. Gatlin, R. Goldburg, K. Hua, and P. Nichols. 2009. Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences of the United States of America* 106: 15103–15110.

Niness, K.R. 1999. Inulin and oligofructose: What are they? *Journal of Nutrition* 129: 1402S–1406S.

Nisbet, D. 2002. Defined competitive exclusion cultures in the prevention of enteropathogen colonization in poultry and swine. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 81: 481–486.

Noda, S., Y. Hongoh, T. Sato, and M. Ohkuma. 2009. Complex coevolutionary history of symbiotic *Bacteroidales* bacteria of various protists in the gut of termites. *BMC Evolutionary Biology* 9: 158. doi:10.1186/1471-2148-9-158.

Odelson, D.A., and J.A. Breznak. 1983. Volatile fatty acid production by the hindgut microbiota of xylophagous termites. *Applied and Environmental Microbiology* 45: 1602–1613.

Odenyo, A.A., R.I. Mackie, D.A. Stahl, and B.A. White. 1994. The use of 16S rRNA targeted oligonucleotide probes to study competition between ruminal fibrolytic bacteria: Pure-culture studies with cellulose and alkaline peroxide-treated wheat straw. *Applied and Environmental Microbiology* 60: 3697–3703.

Orban, J.I., J.A. Patterson, A.L. Sutton, and G.N. Richards. 1997. Effect of sucrose thermal oligosaccharide caramel, dietary vitamin-mineral level, and brooding temperature on growth and intestinal bacterial populations of broiler chickens. *Poultry Science* 76: 482–490.

Peterson, B.C., T.C. Bramble, and B.B. Manning. 2010. Effects of Bio-Mos® on growth and survival of channel catfish challenged with *Edwardsiella ictaluri*. *Journal of the World Aquaculture Society* 41: 149–155.

Pinto-Tomas, A.A., M.A. Anderson, G. Suen, D.M. Stevenson, F.S.T. Chu, W.W. Cleland, P.J. Weimer, and C.R. Currie. 2010. Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. *Science* 326: 1120–1123.

Plugge, C.M. 2005. Anoxic media design, preparation, and considerations. *Methods Enzymology* 397: 3–16.

Pond, M., D. Stone, and D. Alderman. 2006. Comparison of conventional and molecular techniques to investigate the intestinal microflora of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 261: 194–203.

Prescott, L.M., J.P. Harley, and D.A. Klein. 1996. *Microbiology*, 3rd ed. Dubuque: Wm. C. Brown Publishers.

Ramirez, R.F., and B.A. Dixon. 2003. Enzyme production by obligate intestinal anaerobic bacteria isolated from oscars (*Astronotus ocellatus*), angelfish (*Pterophyllum scalare*) and southern flounder (*Paralichthys lethostigma*). *Aquaculture* 227: 417–426.

Rehman, H.U., W. Vahjen, W.A. Awad, and J. Zentek. 2007. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Archives of Animal Nutrition* 61: 319–335.

Ricke, S.C., and S.D. Pillai. 1999. Conventional and molecular methods for understanding probiotic bacteria functionality in gastrointestinal tracts. *Critical Reviews in Microbiology* 25: 19–38.

Ricke, S.C., and D.M. Schaefer. 1990. An ascorbate-reduced medium for nitrogen metabolism studies with *Selenomonas ruminantium*. *Journal of Microbiological Methods* 11: 219–227.

Ricke, S.C., C.L. Woodward, Y.M. Kwon, L.F. Kubena, and D.J. Nisbet. 2004. Limiting avian gastrointestinal tract *Salmonella* colonization by cecal anaerobic bacteria, and a potential role for methanogens. In *Preharvest and postharvest food safety, contemporary issues and future directions*, ed. R.C. Beier, S.D. Pillai, and T.D. Phillips. Ames: Blackwell Publishing Professional.

Rimmer, D.W., and W.J. Wiebe. 1987. Fermentative microbial digestion in herbivorous fishes. *Journal of Fish Biology* 31: 229–236.

Ringo, E. 1993. Does dietary linoleic acid affect intestinal microflora in Artic Charr, *Salvelinus alpinus* (L.)? *Aquaculture Fisheries Management* 24: 133–135.

Ringo, E., R.E. Olsen, T.O. Gifstad, R.A. Dalmo, H. Amlund, G.-I. Hemre, and A.M. Bakke. 2010. Prebiotics in aquaculture: A review. *Aquaculture Nutrition* 16: 117–136.

Ringo, E., H. Bendiksen, S. Gausen, A. Sundsfjord, and R. Olsen. 1998. The effect of dietary fatty acids on lactic acid bacteria associated with the epithelial mucosa and from faecalia of Artic charr, *Salvelinus alpinus* (L.). *Journal of Applied Microbiology* 85: 855–864.

Ringo, E., S. Sperstad, R. Myklebust, T.M. Mayhew, and R.E. Olsen. 2006. The effect of dietary inulin on bacteria associated with hindgut of Artic charr (*Salvelinus alpinus* L.). *Aquaculture Research* 37: 891–897.

Robinson, E.H., H.L. Menghe, and B.B. Manning. 2001. A practical guide to nutrition, feeds and feeding of catfish (Second Revision). Office of Agricultural Communications: Bulletin 1113. http://msucares.com/pubs/bulletins/b1113.pdf. Accessed Jan 5, 2010.

Rohde, F.C., and R.G. Arndt. 1994. *Freshwater fishes of the carolinas, virginia, maryland and delaware*, 124. Chapel Hill: University of North Carolina Press.

Rurangwa, E., Y. Delaedt, Z. Geraylou, T. Van De Wiele, C.M. Courtin, J.A. Delcour, and F. Ollevier. 2008. *Dietary effect of arabinoxylan oligosaccharides on zootechnical performance and hindgut microbial fermentation in Siberian sturgeon and African catfish*, 569–570. Krakow: Aquacult Europe.

Sakata, T., H. Sugita, T. Mitsuoka, D. Kakimoio, and H. Kadota. 1980. Isolation and distribution of obligate anaerobic bacteria from the intestines of freshwater fish. *Bulletin of the Japan Society of Scientific Fisheries* 46: 1249–1255.

Sakata, T., H. Sugita, T. Mitsuoka, D. Kakimoio, and H. Kadota. 1981. Characteristics of obligate anaerobic bacteria in the intestines of freshwater fish. *Bulletin of the Japan Society of Scientific Fisheries* 47: 421–427.

Salanitro, J.P., I.G. Blake, and P.A. Muirhead. 1974a. Studies on cecal microflora of commericial broiler chickens. *Applied Microbiology* 28: 439.

Salanitro, J.P., I.G. Fairchil, and Y.D. Zgonick. 1974b. Isolation, culture characteristics, and identification of anaerobic bacteria from chicken cecum. *Applied Microbiology* 27: 628.

Salanitro, J.P., I.G. Blake, P.A. Muirhead, M. Maglio, and J.R. Goodman. 1978. Bacteria isolated from duodenum, ileum, and cecum of young chicks. *Applied and Environmental Microbiology* 35: 782–790.

Schleifer, K.H. 2009. Classification of bacteria and archaea: Past, present and future. *Systematic and Applied Microbiology* 32: 533–542.

Seiderer, L.J., C.L. Davis, F.T. Robh, and R.C. Newell. 1987. Digestive enzymes of the anchovy *Engraulis capensis* in relation to diet. *Marine Ecology Progress Series* 35: 15–23.

Shelby, R.A., C. Lim, M. Yildirim-Aksoy, and P.H. Klesius. 2007. Effects of probiotic bacteria as dietary supplements on growth and disease resistance in young channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Applied Aquaculture* 19: 81–91.

Shermer, C.L., K.G. Maciorowski, C.A. Bailey, F.M. Byers, and S.C. Ricke. 1998. Caecal metabolites and microbial populations in chickens consuming diets containing a mined humate compound. *Journal of the Science of Food and Agriculture* 77: 479–486.

Shi, Y., and P.J. Weimer. 1997. Competition for cellobiose among three predominant ruminal cellulolytic bacteria under substrate-excess and substrate-limited conditions. *Applied and Environmental Microbiology* 63: 743–748.

Shi, Y., C.L. Odt, and P.J. Weimer. 1997. Competition for cellulose among three predominant ruminal cellulolytic bacteria under substrate-excess and substrate-limited conditions. *Applied and Environmental Microbiology* 63: 734–742.

Smith, T.D. 1992. The isolation and characterization of the cellulolytic anaerobic gastrointestinal microflora of the pinfish (*Lagodon rhomboides*). Masters thesis. Greenville: East Carolina University.

Smith, T.B., D.H. Wahl, and R.I. Mackie. 1996. Volatile fatty acids and anaerobic fermentation in temperate piscivorous and omnivorous freshwater fish. *Journal of Fish Biology* 48: 829–841.

Spanggaard, B., I. Huber, J. Neilson, T. Neilsen, K. Appel, and L. Gram. 2000. The microflora of rainbow trout intestine: A comparison of traditional and molecular identification. *Aquaculture* 182: 1–15.

Staykov, Y., P. Spring, S. Denev, and J. Sweetman. 2007. Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture International* 15: 153–161.

Stellwag, E.J., T.D. Smith, and J.J. Luczkovich. 1995. Characterization and ecology of carboxymethylcellulase-producing anaerobic bacterial communities associated with the intestinal tract of the pinfish, *Lagodon rhomboides*. *Applied and Environmental Microbiology* 61: 813–816.

Stickney, R.R. 1993. *Culture of nonsalmonid freshwater fishes*, 2nd ed. Boca Raton: CRC Press.

Stickney, R.R., and S.E. Shumway. 1974. Occurrence of cellulase activity in the stomachs of fishes. *Journal of Fish Biology* 6: 779–790.

Stoner, A.W., and R.J. Livingston. 1984. Ontogenetic patterns in diet and feeding morphology in sympatric sparid fishes from seagrass meadows. *Copeia* 1984: 174–187.

Strassert, J., M. Desai, R. Radek, and A. Brune. 2010. Identification and localization of the multiple bacterial symbionts of the termite gut flagellate *Joenia annectens*. *Microbiology* 156: 2068–2079.

Sugita, H., K. Tokuyama, and Y. Deguchi. 1985. The intestinal microflora of carp *Cyprinus carpio* grass carp *Ctenopharyngodon idella* and tilapia *Sarothe rodon niloticus*. *Bulletin of the Japan Society of Scientific Fisheries* 51: 1325–1329.

Sugita, H., J. Kawasaki, and Y. Deguchi. 1997. Production of amylase by the intestinal microflora in cultured freshwater fish. *Letters in Applied Microbiology* 24: 105–108.

Sugita, H., K. Ohta, A. Kuruma, and T. Sagesaka. 2007. Short Communication: An antibacterial effect of *Lactococcus lactis* isolated from the intestinal tract of the Amur catfish, *Silurus asotus* Linnaeus. *Aquaculture Research* 38: 1002–1004.

Summanen, P.H., M. McTeague, M.-L. Väisänen, C.A. Strong, and S.M. Finegold. 1999. Comparison of recovery of anaerobic bacteria using the Anoxomat®, anaerobic chamber, and GasPak® jar systems. *Anaerobe* 5: 5–9.

Teitelbaum, J.E., and W.A. Walker. 2002. Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. *Annual Review of Nutrition* 22: 107–138.

Titus, E., and G. Ahearn. 1991. Transintestinal acetate transport in an herbivorous teleost: Anion exchange at the basolateral membrane. *Journal of Experimental Biology* 156: 41–61.

Trust, T.J., L.M. Bull, B.R. Currie, and J.T. Buckley. 1979. Obligate anaerobic bacteria in the gastrointestinal microflora of the grass carp (*Ctenopharyngodon idella*), goldfish (*Caassius auratus*),

and rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* 36: 1174–1179.

Van Hai N., and R. Fotedar. 2009. Comparison of the effects of the prebiotics (Bio-Mos® and β-1,3-D-glucan) and the customized probiotics *(Pseudomonas synxantha* and *P. aeruginosa)* on the culture of juvenile western king prawns *(Penaeus latisulcatus Kishinouye, 1896)* Aquaculture 289: 310–316.

Vandamme, P., B. Pot, M. Gillis, P. De Vos, K. Kersters, and J. Swings. 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiological Reviews* 60: 407–438.

Walden, H.T. 1964. *Familiar freshwater fishes of America*. New York: Harper and Row.

Webster, C.D., J.H. Tidwell, L.S. Goodgame, D.H. Yancey, and L. Mackey. 1992. Use of soybean meal and distillers grains with solubles as partial or total replacement of fish meal in diets for channel catfish, *Ictalurus punctatus*. *Aquaculture* 106: 301–309.

Welker, T.L., C. Lim, M. Yildirim-Aksoy, R. Shelby, and P.H. Klesius. 2007. Immune response and resistance to stress and *Edwardsiella ictaluri* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell yeast or yeast subcomponents. *Journal of the World Aquaculture Society* 38: 24–35.

Wertz, J.T., and J.A. Breznak. 2007. Physiological ecology of *Stenoxybacter acetivorans*, an obligate microaerophile in termite guts. *Applied and Environmental Microbiology* 73: 6829–6841.

Xu, B., Y. Wang, J. Li, and Q. Lin. 2009. Effects on prebiotic xylooligosaccharides on growth performance and digestive activities of allogynogenetic crucian carp (*Carassius auratus gibelio*). *Fish Physiology and Biochemistry* 35: 351–357.

Chapter 12
Use of Direct-Fed Microbials as a Preharvest Food Safety Intervention in Cattle

Megan E. Jacob and T.G. Nagaraja

Abstract Each year an estimated 76 million people in the United States develop a food-borne illness. Often, food animals are reservoirs for bacterial food-borne agents; these organisms are frequently part of the normal flora in the animals' gastrointestinal tract and can be shed in the feces subsequently contaminating the environment or carcass at harvest. The USDA Food Safety and Inspection Service currently sets guidelines and oversees standards to reduce the risk of bacterial contamination of meat and poultry products, yet illnesses still occur. The discovery of new routes of pathogen transmission on items such as produce highlight the importance of additional mitigation strategies in food animals prior to harvest. Preharvest interventions to reduce the prevalence and concentration of food-borne pathogens have become a major research priority. These products include direct-fed microbials (DFMs). This chapter reviews the evidence for the use of DFMs to reduce food-borne pathogenic bacteria in cattle before they enter the food chain.

12.1 Introduction

Each year an estimated 76 million people in the United States develop a food-borne illness (CDC 2010). These illnesses range in severity from asymptomatic or mild gastrointestinal (GI) upset to death. The populations at highest risk for severe illness or death are frequently young children, the elderly, or immunocompromised persons. A number of bacterial, viral, and parasitic agents have been associated with food-borne illnesses, and these organisms can be transmitted on a seemingly endless array of food products and in water (Mead et al. 1999; Lynch et al. 2009). Often,

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food animals are reservoirs for bacterial food-borne agents; and these organisms are frequently part of the normal flora in the animals' GI tract. They can be shed in the feces, contaminating the environment or carcass at harvest. The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) currently sets guidelines and oversees standards to reduce the risk of bacterial contamination of meat and poultry products (USDA, FSIS 2010a), yet illnesses still occur. The discovery of new routes of pathogen transmission on items such as produce highlight the importance of additional mitigation strategies in food animals prior to harvest. Preharvest interventions to reduce the prevalence and concentration of food-borne pathogens have become a major research priority. Products such as direct-fed microbials (DFMs), vaccines, bacteriophage, and antimicrobials have been proposed as preharvest interventions in food animals (Callaway et al. 2004; Loneragan and Brashears 2005; LeJeune and Wetzel 2007).

The beneficial effects of ingesting certain microorganisms or their products have been recognized since the early twentieth century when Metchnikoff demonstrated that lactic acid bacteria from fermented foods could have human health benefits. Still, only during the past several decades has there been a resurgence in the use and applicability of such products commercially. DFMs now have applications in food animals with a variety of desirable outcomes. In addition to products being used for performance benefits and GI health, one developing use is competitive exclusion of bacterial food-borne pathogens. To date, this use has primarily been evaluated in cattle and poultry, both of which are capable of shedding pathogenic organisms in their feces, potentially contaminating food, water, and the environment. In such an application, the DFM product would reduce the prevalence or concentration of food-borne pathogens in animals prior to their harvest, thereby reducing the potential pathogen load in the environment and at the abattoir. Most of the products currently evaluated for such an application in cattle were examined for their efficacy in reducing the *Escherichia coli* O157:H7 concentration and/or prevalence. Applications against other food-borne organisms, including non-O157 Shiga toxin-producing *E. coli* (STEC) and *Salmonella*, are also being developed. In poultry, the focus has been on reduction of *Salmonella* and *Campylobacter*. The following sections provide a review of published work on competitive exclusion of food-borne pathogens in cattle. (The role of DFMs in poultry production and food safety are discussed elsewhere in this book). There is also a brief discussion of the potential mechanisms responsible for pathogen reduction, focused on *E. coli* O157:H7.

12.2 Preharvest Food Safety in Cattle

Cattle are reservoirs for several bacterial food-borne pathogens, including *E. coli* O157:H7 and other STEC, *Salmonella enterica*, *Campylobacter jejuni*, and *Listeria monocytogenes* (Callaway et al. 2006; Hannon et al. 2009). Often, these bacteria colonize the lower GI tract of animals asymptomatically. Cattle may become colonized with these organisms early in the animal's life, probably within weeks of birth

(Gannon et al. 2002; Pearce et al. 2004). Perhaps the most important food-borne pathogen to the U.S. cattle industry is *E. coli* O157:H7. Traditionally, this organism has been associated with ground beef, although other food products, including produce, may be transmission vehicles (Rangel et al. 2005). Each year an estimated 73,000 human cases of *E. coli* O157:H7 infections occur; and the pathogen, which is considered an adulterant, was associated with over 43 million pounds of beef product recalls between 2007 and 2009 (USDA, FSIS 2010b). The USDA federal testing and inspection requirements, as well as a number of postharvest interventions, reduce the risk of *E. coli* O157:H7 and other contaminants entering the food chain. Still, new food products are being identified as transmission vehicles, and their contamination routes might not be as obvious. There is a need for preharvest interventions in cattle to reduce the carriage of *E. coli* O157:H7 and other pathogens that could be transmitted to other animals throughout the environment or serve as a source of reinoculation. If pathogen colonization and concentration in the animal is reduced, it is likely that the number of food-borne outbreaks would also diminish.

Competitive exclusion was first described by Nurmi in Finland and was in the context of providing normal microbial inhabitants that could protect against *Salmonella* colonization in the chick GI tract (Nurmi and Rantala 1973). This term is now used to describe the reduction of other pathogens, including *E. coli* O157:H7, in cattle when animals are orally administered normal GI tract microorganisms. DFMs are becoming increasingly popular in cattle production as a nonantimicrobial alternative to improve performance and health (Krehbiel et al. 2003). Frequently strains or fermentation products of *Lactobacillus*, *Enterococcus*, commensal *E. coli*, and *Bifdobacterium* species are used as DFMs, although some fungal products have also been evaluated. Most of the reported literature for DFM use in cattle evaluates products containing strains of commensal *E. coli* or *Lactobacillus*. Several commercially available products are now starting to promote the competitive exclusion capacity of their DFM to reduce *E. coli* O157:H7 in addition to other health and performance benefits. If these products can competitively exclude *E. coli* O157:H7, *Salmonella*, or other pathogens, they may become an extremely useful preharvest intervention tool. However, none of the commercial products currently available have approved claims for pathogen control or reduction.

12.3   Direct-Fed Microbial Products Containing Commensal *E. coli*

The use of commensal *E. coli* to competitively exclude *E. coli* O157:H7 and other STECs seems intuitive and has been evaluated in experimental challenge studies. Previous work in mouse models has shown that precolonization of the GI tract with commensal *E. coli* organisms can protect against *E. coli* O157:H7 colonization (Gamage et al. 2006; Leatham et al. 2009). Protection against *E. coli* O157:H7 or other STEC colonization in cattle has not been as well described; and the efficacy in reducing fecal shedding of these organisms is inconsistent. In 24-h laboratory fermentations with cattle rumen or a fecal inoculum, *E. coli* competitive

Table 12.1 Experimental challenge studies evaluating the fecal prevalence of STEC in cattle fed DFMs containing commensal *Escherichia coli* species

STEC evaluated	Cattle population	Result	Reference
E. coli O157:H7	Holstein calves	Fecal concentration significantly* less in the treatment group compared to controls at the end of the study (18 days after challenge)	Zhao et al. (1998)
E. coli O157:H7	Holstein calves	No significant difference between treatments	Zhao et al. (2003)
E. coli O111:NM	<1 week old	Probiotic treatment shed less on days 3 and 7 after infection than control calves	
E. coli O26:H11		Probiotic treatment shed less on days 6 and 7 after infection than control calves	
E. coli O157:H7	Holstein calves	Fecal shedding significantly reduced in calves fed probiotic on 8 of 16 sampling days	Tkalcic et al. (2003)
E. coli O111:NM		Fecal shedding significantly reduced in calves fed probiotic on 4 of 16 sampling days	
E. coli O26:H11		No significant difference between treatments	
E. coli O157:H7	Holstein calves	No statistical difference in fecal concentration between DFM and control treatments within a study phase	Schamberger et al. (2004)

STEC Shiga toxin-producing *E. coli*, *DFM* direct-fed microbial
*Statistical significance is considered at $P < 0.05$

exclusion cultures were associated with a lower *E. coli* O157:H7 concentration than in nontreated fermentations (Fox et al. 2009). These in vitro competitive exclusion experiments were done using "probiotic" *E. coli* isolates identified by Zhao et al. (1998). Zhao et al. (1998) screened approximately 1,200 bacterial isolates obtained from *E. coli* O157:H7-negative cattle feces for their potential to inhibit the organism. In vitro inhibitory effects were observed for 17 *E. coli* isolates, which were confirmed to be negative STECs and were evaluated for lack of pathogenicity and probiotic potential in calves. Administration of the isolated *E. coli* bacteria prior to oral challenge with *E. coli* O157:H7 reduced carriage of the *E. coli* O157:H7 for most study animals (Zhao et al. 1998). Similar results were reported for a related experimental challenge study with fecal STEC serotypes O26:H11 and O111:NM (Zhao et al. 2003) (Table 12.1). Fecal shedding of these two STECs was reduced in young calves (<1 week of age) pretreated with a three-strain mixture of *E. coli* (obtained from the previous study). In this study, there was no significant reduction in *E. coli* O157:H7 shedding between treated and untreated calves. In older, weaned calves, the STEC serotype response to treatment with competitive exclusion *E. coli* was different (Tkalcic et al. 2003). Here, the O26:H11

concentration was not reduced in probiotic-treated calves, although *E. coli* O111:NM and O157:H7 shedding was reduced for several collection days. These competitive exclusion *E. coli* cultures have not consistently reduced the shedding of *E. coli* O157:H7, O26:H11, or O111:NM on all shedding days in this series of trials. However, cattle were administered the *E. coli* treatment only one time, either before or after inoculation; in practice, a DFM product would likely be administered daily. The effect of continual dosing of commensal *E. coli* on fecal shedding of STEC has not been reported. In a separate study, *E. coli* strains previously shown to produce bacteriocins, called colicins, were fed to cattle prior to experimental challenge with *E. coli* O157:H7 (Schamberger et al. 2004). Although this product did exhibit trends for lower *E. coli* O157:H7 fecal concentrations in calves receiving the DFM product compared to control calves, the differences were not statistically significant. There have been no published reports of trials in natural prevalence feedlot conditions using an *E. coli*-based DFM product, making it difficult to assess the ability of these products to serve as a preharvest intervention in the commercial setting.

12.4   Direct-Fed Microbial Products Containing Lactic Acid Bacteria

The ability of lactic acid bacteria, most commonly *Lactobacillus* species, to reduce the prevalence or concentration of food-borne pathogens in cattle has been more extensively reported than DFM products with any other bacteria (Table 12.2). Lactic acid bacteria as a group are Gram-positive rods or cocci that are metabolically similar regarding fermenting carbohydrates to produce lactic acid as a major end-product. Lactobacilli are part of the normal flora of the rumen and the lower gut. Although the use of lactobacilli as a food-borne pathogen intervention in cattle is relatively new, the organisms have been in use as feed additives to modify ruminal function and improve animal performance (Krehbiel et al. 2003).

   A screen of lactic acid bacteria from cattle feces culture negative for *E. coli* O157:H7 revealed a significant number of isolates that could inhibit the organism in vitro (Brashears et al. 2003a). After evaluating isolates for acid and bile tolerance, antimicrobial susceptibility, and efficacy at reducing *E. coli* O157:H7 in fecal and rumen fluid samples, the authors found *Lactobacillus* species with anti-*E. coli* O157 efficacy and potential as a competitive exclusion product. Since then, the same group has published several natural prevalence feedlot trials that showed a reduction in the fecal shedding of *E. coli* O157:H7 in cattle administered the *L. acidophilus* product as a DFM (combined with a *Propionibacterium freudenreichii* species) (Brashears et al. 2003b; Elam et al. 2003; Younts-Dahl et al. 2004), an association that initially appeared to be linearly associated with the DFM dose (Younts-Dahl et al. 2005). Stephens et al. (2007a) reported a reduced likelihood of *E. coli* O157:H7 recovery from cattle feces or hides if the animal was administered *L. acidophilus* NP51, although in their trial there was no linear association with the

Table 12.2 Natural prevalence studies evaluating fecal prevalence of food-borne pathogens in cattle[a] fed DFMs containing *Lactobacillus* species

Food-borne pathogen	DFM	Cattle Population	Result	Reference
E. coli O157:H7	*L. acidophilus* NP51, NP45	Finishing cattle	Cattle receiving 10^9 cfu/steer daily NP51 had reduced fecal prevalence compared to controls receiving no DFM	Brashears et al. (2003b)
E. coli O157:H7	*L. acidophilus* NP51, NP 45 *Propionibacterium freudenreichii*	Finishing cattle	Cattle receiving 10^9 cfu/steer daily NP51 had reduced fecal prevalence compared to controls receiving no DFM	Elam et al. (2003)
E. coli O157:H7	*L. acidophilus* NP51, NP45 *P. freudenreichii*	Finishing cattle / Finishing cattle	Cattle receiving 10^9 cfu/steer daily NP51 had reduced fecal prevalence compared to controls receiving no DFM	Younts-Dahl et al. (2004)
E. coli O157:H7	*L. acidophilus* NP51, NP45 *P. freudenreichii*	Finishing cattle	Reduced fecal prevalence for increasing concentrations of NP51 fed throughout study period	Younts-Dahl et al. (2005)
E. coli O157	*L. acidophilus* NP51 *P. freudenreichii*	Finishing cattle	No statistical* difference between treatments	Woerner et al. (2006)
E. coli O157:H7	*L. acidophilus* NP51	Finishing cattle	Cattle fed high (10^9 cfu/steer) and low (10^7 cfu/steer) NP51 daily had reduced fecal prevalence; no linear association	Stephens et al. (2007a)
Salmonella	*P. freudenreichii*	Finishing cattle	No statistically significant reduction in fecal prevalence	
E. coli O157:H7	*L. acidophilus* NP51, NP28, NP35, or NP51-NP35 *P. freudenreichii*	Finishing cattle	Reduced fecal prevalence in cattle receiving two of three individual strains as DFM products compared to controls	Stephens et al. (2007b)
E. coli O157:H7	*L. acidophilus* NP51 *P. freudenreichii*	Finishing cattle	Cattle receiving 10^9 cfu/steer daily NP51 had reduced fecal prevalence compared to controls receiving no DFM over 2-year trial	Peterson et al. (2007)
E. coli O157:H7	*L. acidophilus* BT1386	Finishing cattle	Reduced fecal prevalence in DFM treated steers compared to controls	Tabe et al. (2008)
Salmonella			No effect on fecal prevalence between treatment and control steers	
E. coli O157:H7	*L. acidophilus*, *L. casei*, *S. cerevisiae*, *E. faecium*, fungus extract	Finishing cattle	Reduced fecal prevalence in cattle administered DFM product compared to controls	Jacob et al. (2010)

*Statistical significance is considered at $P<0.05$

[a]Cattle in all of the studies were finishing cattle

DFM dose. However, at the highest dose, this product was also shown to reduce the *Salmonella* fecal and hide prevalence in cattle during the same trial (Stephens et al. 2007a). Other groups have also evaluated the same *L. acidophilus* DFM product as a preharvest food safety intervention. A 2-year feedlot study found *L. acidophilus* NP51 to be effective in reducing *E. coli* O157:H7 prevalence in feeder cattle (Peterson et al. 2007). Woerner et al. (2006) conducted a study evaluating multiple interventions applied individually or in combination on *E. coli* O157 prevalence in feces and hides. They found a trend for decreased prevalence in both sample types when cattle were administered the *L. acidophilus* DFM; treatment in this study was not statistically significant, however, as there were only three pens for each treatment group. Finally, Tabe et al. (2008) reported the efficacy of a different *L. acidophilus* DFM strain (BT1386) for reducing *E. coli* O157:H7, but not *Salmonella*, prevalence in yearling feedlot steers. This strain of *L. acidophilus* has also been shown to inhibit *E. coli* O157:H7 in a dose-dependent response using in vitro fecal suspensions (Chaucheyras-Durand et al. 2006).

Several feeding trials have reported efficacy of DFM products with multiple lactic acid bacterial strains combined with or without additional organisms aimed at *E. coli* O157:H7 reduction. One product containing *L. acidophilus*, *L. casei*, *Saccharomyces cerevisiae*, *Enterococcus faecium*, and a fungal extract was shown to reduce the prevalence of *E. coli* O157:H7 in feedlot cattle when the product was fed to them for 15 days immediately prior to slaughter (Jacob et al. 2010). A similar mixture of bacterial and fungal organisms has been evaluated in sheep, which can also be a reservoir for *E. coli* O157:H7 and often serves as a model for cattle. The combination of *L. acidophilus*, *L. casei*, *L. fermentum*, and *L. plantarum* combined with *E. faecium* reduced *E. coli* O157:H7 shedding in experimentally challenged lambs when compared to other DFM or control treatments (Lema et al. 2001). Clearly, there are several DFM products containing *Lactobacillus* organisms that appear effective at reducing *E. coli* O157:H7 and possibly *Salmonella* in feedlot cattle. However, previous work suggests that not all *Lactobacillus* strains or DFM products reduce the concentration and prevalence or inhibit the organism in vitro (Brashears et al. 2003a; Stephens et al. 2007b). The reasons for differences in the degree to which shedding is reduced and the success of some products but not others remain unknown, but it may be associated with the mechanism of action specific to each bacterial strain.

12.5 Direct-Fed Microbial Products Containing Other Organisms

Occasionally, DFM products that contain organisms other than lactic acid bacteria or *E. coli* have been evaluated for efficacy as a preharvest *E. coli* O157:H7 competitive exclusion intervention. Arthur et al. (2010) evaluated a *Bacillus subtilis* strain as a DFM and found no effect on *E. coli* O157:H7 prevalence or on the concentration in feces or on hides. In a randomized controlled trial of feedlot cattle, neither

Saccharomyces cerevisiae subsp. *boulardii* nor *Aspergillus oryzae* products were associated with the *E. coli* O157:H7 prevalence independent of grain type (Cernicchiaro et al. 2010). The *S. cerevisiae* subsp. *boulardii* was shown to have anti-*E. coli* O157:H7 action on spot agar assays, but with rumen fluid inoculations there was no effect of the DFM on the *E. coli* O157:H7 concentration (Bach et al. 2003). It seems likely that proprietary products with bacterial strains believed to improve cattle health and performance may also be evaluated for their potential to reduce the prevalence of *E. coli* O157:H7 or other food-borne pathogens. It is difficult to predict the success and mechanisms of action of these products, and to date there is no evidence of efficacy for any of these products in a natural prevalence, clinical trial setting.

12.6 Potential Mechanisms

It is clear that most of the DFM products evaluated for food-borne pathogen intervention in cattle target *E. coli* O157:H7, likely because of the importance of this organism to the beef industry. Therefore, the potential mode of action of DFM products here focuses on *E. coli* O157:H7 reduction, although one would expect similar mechanisms for other organisms, particularly the non-O157 STEC. Traditionally, inhibition of food-borne pathogens by DFMs are broadly grouped into indirect effects mediated by changes in the microbial ecosystem in the GI tract and direct effects where DFM organisms exert effects on the food-borne pathogens. More specifically, production of antibacterial substances, competition for attachment or colonization sites in the GI tract, and immunomodulation are mechanisms by which DFM organisms can inhibit other bacteria (Brashears et al. 2005). There is evidence to support that all of these mechanisms may be useful for reducing *E. coli* O157:H7 in cattle. Although other mechanisms such as reduced expression of virulence genes in *E. coli* O157:H7 and *Salmonella* by secreted molecules of probiotic organisms have also been reported (Medellin-Peña et al. 2007; Bayoumi and Griffiths 2010), their role in reducing pathogen prevalence or concentration in food animals has not been evaluated.

Studying the mechanism of a DFM product in cattle is complicated by the interactions and passage through the rumen. The food-borne pathogens shed in the feces of cattle colonize in the lower GI tract. Thus, passage through the GI tract and normal function and health is crucial to the competitive exclusion potential of any DFM. However, it is often not known if these organisms have the ability to survive and proliferate in the hindgut, where they would inhibit *E. coli* O157:H7.

12.6.1 Production of Antibacterial Products

Bacteria produce several antibacterial substances that can inhibit competing flora, including hydrogen peroxide, organic acids, and bacteriocins. The effects of these

products on *E. coli* O157:H7 or other bacteria have primarily been evaluated in vitro. The production and concentration of the products in the animal is complicated by other factors. Oxidative products are frequently produced by bacteria and may influence the gut microbial ecosystem. Strains of intestinal *Lactobacillus* have been found to produce varying levels of hydrogen peroxide, which is a known antibacterial substance (Annuk et al. 2003). Production of hydrogen peroxide or other similar products in the intestinal tract from administered DFM organisms has not been reported; however, if it were produced, it would likely not be selective for specific food-borne organisms. It is more likely there would be an effect on the normal flora, which may indirectly have an impact on the prevalence or concentration of specific bacteria. This may be true for other antibacterial substances as well, including organic acids.

Bacteria in the large intestine of animals often produce organic acids as metabolic end-products. Total acid production is related to the dietary substrates available, host factors (rate of absorption), and changes in the microbial populations. The specific acids produced are not the only ones affected by the host and environment, so are the bacteria – and it may even be strain-specific. End-products are generally short-chain fatty acids including acetate, propionate, butyrate, and lactate (Macfarlane and Macfarlane 2003). In vitro experiments showed strains of *Lactobacillus* that were also capable of producing succinic acid, and some strains could produce two to three times more than others (Annuk et al. 2003). Still, the primary fermentation product of lactic acid bacteria is lactate (although other products may be produced), and other DFMs containing *Propionibacterium* should produce propionate. The in vivo production and concentration of these acids by the organisms in DFM products is not known. Still, if these organisms behave and function normally in the lower gut, we would expect organic acid production. Previous work with *E. coli* O157:H7 has shown that the organism is sensitive to lactic acid; however, inhibition is pH-dependent and augmented by ethanol, another product of lactobacilli (Jordan et al. 1999). Similar findings were reported for acetic, propionic, butyric, and lactic acids at lower pH (Shin et al. 2002). In addition, numerous studies have evaluated these acids for reducing the viability of *E. coli* O157:H7 in food products. McWilliam Leitch and Stewart (2002) showed that lactate and propionate were effective at reducing the viability of *E. coli* O157:H7 and other STEC in vitro, although inhibition was temperature-specific. Work in our laboratory has shown that *E. coli* O157:H7 can be inhibited by *L. acidophilus* enrichment, although if the pH of the enrichment is brought to neutral (~7), there is no observed inhibition (unpublished data). In vitro fecal suspensions treated with *L. acidophilus* exhibited a decrease in *E. coli* O157:H7, pH, lactate concentration, and total lactic acid bacteria counts compared to control fermentations (Chaucheyras-Durand et al. 2006). In an infant rabbit model, animals treated with *L. casei* and challenged with *E. coli* O157:H7 did not differ in fecal pH and had only a slightly higher fecal lactic acid concentration than challenged animals not given the DFM (Ogawa et al. 2001). It seems unlikely that the pH of the hindgut would be low enough to duplicate the in vitro effect (pH < 6); however, some reduction of pH, particularly in the microenvironment of the pathogen, may contribute to overall fitness or other competitive exclusion mechanisms. Also, the organic acid effect may not necessarily be related

to pH alone but to the pKa of the acid and the ability of the acid to enter bacterial cells and cause damage.

Finally, bacteriocins are small peptides secreted by bacteria that are inhibitory to closely related organisms. *Escherichia coli* commonly produces colicins, which can inhibit closely related *E. coli* strains including *E. coli* O157:H7 (Murinda et al. 1996). This is supported by the work of Zhao et al. (1998), who showed that *E. coli* isolated from the feces of cattle were capable of inhibiting *E. coli* O157:H7 in vitro. *Lactobacillus* species are also capable of producing bacteriocins, but their ability to inhibit Gram-negative organisms such as *E. coli* O157:H7 is unclear (Brashears et al. 2005). These products may be effective in reducing other Gram-positive food-borne pathogens including *Listeria monocytogenes*, however.

12.6.2   Competition for Attachment Sites

A common proposed mechanism for inhibition or reduction of food-borne pathogens in the GI tract is competition for attachment or colonization sites. Colonization of these sites by other normal flora or DFM organisms would in turn protect against colonization from the food-borne pathogens of interest (Brashears et al. 2005). Data to support this mechanism is limited, although some conflicting data exist from monogastric models. Work by Spencer and Chesson (1994) found that *Lactobacillus* strains adherent to porcine enterocytes did little to inhibit enterotoxigenic *E. coli* attachment. Still, in studies with HEp-2 and T84 epithelial cell lines pretreated with *Lactobacillus* species, *E. coli* O157:H7 adherence and epithelial cell injury were reduced compared to that of untreated cells (Sherman et al. 2005). Additionally, molecules secreted by *L. acidophilus* were shown to reduce adherence and lesions from *E. coli* O157:H7 in HEp-2 and HeLa cell lines and inhibited expression of genes associated with *E. coli* O157:H7 attachment (Medellin-Peña et al. 2007; Medellin-Peña and Griffiths 2009). This reduction may be related to the ability of *E. coli* O157:H7 to communicate by interrupting quorum-sensing molecules that are important for gene expression. More work is needed to understand the role of competition for attachment in the ruminant hindgut.

12.6.3   Immunomodulation

Stimulation of the immune system by DFM organisms may contribute to inhibition or reduction of food-borne pathogens in the GI tract. Either a humoral response by immunoglobulins A (IgA) and IgM associated with the mucus membrane or nonhumoral immunity (e.g., increased phagocytic activity, cytokine production) may contribute to altered microbial ecology. There is evidence in mouse and rabbit models that *Lactobacillus* species stimulate the immune system against *E. coli* O157:H7 and *Salmonella* infections. In infant rabbits inoculated with *E. coli* O157:H7, the

severity of infections and colonization was reduced if treated daily with an *L. casei* strain (Ogawa et al. 2001). In addition, at 7 days after the infection, there was evidence of a significant increase in the anti-Stx1 and Stx2 IgA response, supporting local immunomodulation. Similarly, in mice treated for a week with heat-killed, mixed *L. acidophilus* strains and challenged with *Salmonella typhimurium*, colonization was reduced and there was evidence of immunomodulation (Lin et al. 2007). Still, in these studies the challenge organisms were given at high doses, and both *E. coli* O157:H7 and *Salmonella typhimurium* are pathogenic to mice, which is not the case in cattle. The specific immune response in the established bovine GI tract against *E. coli* O157:H7 or *Salmonella* may not be as substantial or compelling.

12.7   Conclusions

Direct-fed microbials are increasing in popularity, partially driven by the need for nonantimicrobial alternatives to improved animal health and performance. An additional advantage for these microbial additives may be the reduction of pathogenic food-borne bacteria. Several products may be effective in reducing the prevalence and concentration of *E. coli* O157:H7 in cattle, but their efficacy in reducing other food-borne pathogens remains unknown. The precise mechanism responsible for reducing the presence of any organism by competitive exclusion may not be known, but it is likely specific to each DFM product and each organism inhibited. There is evidence supporting the idea that organisms frequently included in DFMs can produce antibacterial substances, compete for attachment sites, and induce immunomodulation responses in the GI tract, all of which could inhibit food-borne pathogens. More research is needed to identify specific mechanisms associated with this reduction. The results of such studies may lead to the development of more efficacious DFM products.

References

Annuk, H., J. Shchepetova, T. Kullisaar, et al. 2003. Characterization of intestinal lactobacilli as putative probiotic candidates. *Journal of Applied Microbiology* 94: 403–412.

Arthur, T.M., J.M. Bosilevac, N. Kalchayanand, et al. 2010. Evaluation of a direct-fed microbial product effect on the prevalence and load of *Escherichia coli* O157:H7 in feedlot cattle. *Journal of Food Protection* 73: 366–371.

Bach, S.J., T.A. McAllister, D.M. Veira, et al. 2003. Effects of a *Saccharomyces cerevisiae* feed supplement on *Escherichia coli* O157:H7 in ruminal fluid in vitro. *Animal Feed Science and Technology* 104: 179–189.

Bayoumi, M.A., and M.W. Griffiths. 2010. Probiotics down-regulate genes in *Salmonella enteric* serovar Typhimurium pathogenecity islands 1 and 2. *Journal of Food Protection* 73: 452–460.

Brashears, M.M., D. Jaroni, and J. Trimble. 2003a. Isolation, selection and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. *Journal of Food Protection* 66: 355–363.

Brashears, M.M., M.L. Galyean, G.H. Loneragan, et al. 2003b. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *Journal of Food Protection* 66: 748–754.

Brashears, M.M., A. Amezquita, and D. Jaroni. 2005. Lactic acid bacteria and their uses in animal feeding to improve food safety. *Advances in Food and Nutrition Research* 50: 1–31.

Callaway, T.R., R.C. Anderson, T.S. Edrington, et al. 2004. Recent pre-harvest supplementation strategies to reduce carriage and shedding of zoonotic enteric bacterial pathogens in food animals. *Animal Health Research Reviews* 5: 35–47.

Callaway, T.R., T.S. Edrington, A.D. Brabban, et al. 2006. Fecal prevalence of *Escherichia coli* O157, *Salmonella*, *Listeria*, and bacteriophage infecting *E. coli* O157:H7 in feedlot cattle in the southern plains region of the United States. *Foodborne Pathogens and Disease* 3: 234–244.

Centers for Disease Control and Prevention, 2010. *Food safety*. Available at: http://www.cdc.gov/foodsafety. Accessed June 9, 2010.

Cernicchiaro, N., D.L. Pearl, S.A. McEwen, H.N. Zerby, F.L. Fluharty, S.C. Loerch, M.D. Kauffman, J.L. Bard, and J.T. LeJeune. 2010. A randomized controlled trial to assess the impact of dietary energy sources, feed supplements, and the presence of super-shedders on the detection of *Escherichia coli* O157:H7 in feedlot cattle using different diagnostic procedures. *Foodborne Pathogens and Disease* 7: 1071–1081.

Chaucheyras-Durand, F., J. Madic, F. Doudin, et al. 2006. Biotic and abiotic factors influencing in vitro growth of *Escherichia coli* O157:H7 in ruminant digestive contents. *Applied and Environmental Microbiology* 72: 4136–4142.

Elam, N.A., J.F. Gleghorn, J.D. Rivera, et al. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. *Journal of Animal Science* 81: 2686–2698.

Fox, J.T., J.S. Drouillard, and T.G. Nagaraja. 2009. Competitive exclusion *Escherichia coli* cultures on *E. coli* O157 growth in batch culture ruminal or fecal microbial fermentation. *Foodborne Pathogens and Disease* 6: 193–199.

Gamage, S.D., A.K. Patton, J.E. Strasser, et al. 2006. Commensal bacteria influence *Escherichia coli* O157:H7 persistence and Shiga toxin production in the mouse intestine. *Infection and Immunity* 74: 1977–1983.

Gannon, V. P., T. A. Graham, R. King, et al. 2002. *Escherichia coli* O157:H7 infection in cows and calves in a beef cattle herd in Alberta, Canada. *Epidemiology and Infection* 129: 163–172.

Hannon, S.J., B. Allan, C. Waldner, et al. 2009. Prevalence and risk factor investigation of *Campylobacter* species in beef cattle feces from seven large commercial feedlots in Alberta, Canada. *Canadian Journal of Veterinary Research* 73: 275–282.

Jacob, M.E., K. Lechtenbreg, L.L. Burnham, et al. 2010. *Feeding MSE direct-fed microbials to finishing cattle decreases the fecal shedding of Escherichia coli O157:H7*. Abstract #318 Midwestern Section ASAS/ADSA, Des Moines, IA. March 2010.

Jordan, S.L., J. Glover, L. Malcolm, et al. 1999. Augmentation of killing of *Escherichia coli* O157 by combinations of lactate, ethanol, and low-pH conditions. *Applied and Environmental Microbiology* 65: 1308–1311.

Krehbiel, C.R., S.R. Rust, G. Zhang, et al. 2003. Bacterial direct-fed microbials in ruminant diets: performance response and mode of action. *Journal of Animal Science* 81: E120–E132.

Leatham, M.P., S. Banerjee, S.M. Autieri, et al. 2009. Precolonized human commensal *Escherichia coli* strains serve as a barrier to *E. coli* O157:H7 growth in the streptomycin-treated mouse intestine. *Infection and Immunity* 77: 2876–2886.

LeJeune, J.T., and A.N. Wetzel. 2007. Preharvest control of *Escherichia coli* O157 in cattle. *Journal of Animal Science* 85: E73–E80.

Lema, M., L. Williams, and D.R. Rao. 2001. Reduction of fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. *Small Ruminant Research* 39: 31–39.

Lin, W., B. Yu, C. Lin, et al. 2007. Immune effect of heat-killed multistrain of *Lactobacillus acidophilus* against *Salmonella typhimurium* invasion to mice. *Journal of Applied Microbiology* 102: 22–31.

Loneragan, G.H., and M.M. Brashears. 2005. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. *Meat Science* 71: 72–78.

Lynch, M.F., R.V. Tauxe, and C.W. Hedberg. 2009. The growing burden of food-borne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection* 137: 307–315.

Macfarlane, S., and G.T. Macfarlane. 2003. Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society* 62: 67–72.

McWilliam Leitch, E.C., and C.S. Stewart. 2002. Susceptibility of *Escherichia coli* O157 and non-O157 isolates to lactate. *Letters in Applied Microbiology* 35: 176–180.

Mead, P.S., L. Slutsker, V. Dietz, et al. 1999. Food-related illness and death in the United States. *Emerging Infectious Diseases* 5: 607–625.

Medellin-Peña, M.J., and M.W. Griffiths. 2009. Effect of molecules secreted by *Lactobacillus acidophilus* strain La-5 on *Escherichia coli* O157:H7 colonization. *Applied and Environmental Microbiology* 75: 1165–1172.

Medellin-Peña, M.J., H. Wang, R. Johnson, et al. 2007. Probiotics affect virulence-related gene expression in *Escherichia coli* O157:H7. *Applied and Environmental Microbiology* 73: 4259–4267.

Murinda, S.E., R.F. Roberts, and R.A. Wilson. 1996. Evaluation of colicins for inhibitory activity against diarrheagenic *Escherichia coli* strains, including serotype O157:H7. *Applied and Environmental Microbiology* 62: 316–3202.

Nurmi, E., and M. Rantala. 1973. New aspects of *Salmonella* infection in broiler production. *Nature* 241: 210–211.

Ogawa, M., K. Shimizu, K. Nomoto, et al. 2001. Protective effect of *Lactobacillus casei* strain Shirota on shiga-toxing producing *Escherichia coli* O157:H7 infection in infant rabbits. *Infection and Immunity* 69: 1101–1108.

Pearce, M.C., C. Jenkins, L. Vali, et al. 2004. Temporal shedding patterns and virulence factors of *Escherichia coli* serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams. *Applied and Environmental Microbiology* 70: 1708–1716.

Peterson, R.E., T.J. Klopfenstein, G.E. Erickson, et al. 2007. Effect of *Lactobacillus acidophilus* strain NP51 on *Escherichia coli* O157:H7 fecal shedding and finishing performance in beef feedlot cattle. *Journal of Food Protection* 70: 287–291.

Rangel, J.M., P.H. Sparling, C. Crowe, et al. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerging Infectious Diseases* 11: 603–609.

Schamberger, G.P., R.L. Phillips, J.L. Jacobs, et al. 2004. Reduction of *Escherichia coli* O157:H7 populations in cattle by addition of colicin E7-producing *E. coli* to feed. *Applied and Environmental Microbiology* 70: 6053–6060.

Sherman, P.M., K.C. Johnson-Henry, H.P. Yeung, et al. 2005. Probiotics reduce enterohemorrhagic *Escherichia coli* O157:H7- and enteropathogenic *E. coli* O127:H6-induced changes in polarized T84 epithelial cell monolayers by reducing bacterial adhesion and cytoskeletal rearrangements. *Infection and Immunity* 73: 5183–5188.

Shin, R., M. Suzuki, and Y. Morishita. 2002. Influence of intestinal anaerobes and organic acids on the growth of enterohaemorrhagic *Escherichia coli* O157:H7. *Journal of Medical Microbiology* 51: 201–206.

Spencer, R.J., and A. Chesson. 1994. The effect of *Lactobacillus* spp. on the attachment of enterotoxigenic *Escherichia coli* to isolated porcine enterocytes. *Journal of Applied Bacteriology* 77: 215–220.

Stephens, T.P., G.H. Loneragan, E. Karunasena, et al. 2007a. Reduction of *Escherichia coli* O157 and *Salmonella* in feces and on hides of feedlot cattle using various doses of a direct-fed microbial. *Journal of Food Protection* 70: 2386–2391.

Stephens, T.P., G.H. Loneragan, L.M. Chichester, et al. 2007b. Prevalence and enumeration of *Escherichia coli* O157 in steers receiving various strains of *Lactobacillus*-based direct-fed microbials. *Journal of Food Protection* 70: 1252–1255.

Tabe, E.S., J. Oloya, D.K. Doetkott, et al. 2008. Comparative effect of direct-fed microbials on fecal shedding of *Escherichia coli* O157:H7 and *Salmonella* in naturally infected feedlot cattle. *Journal of Food Protection* 71: 539–544.

Tkalcic, S., T. Zhao, B. G. Harmon et al. 2003. Fecal shedding of enterohemorrhagic *Escherichia coli* in weaned calves following treatment with probiotic *Escherichia coli*. *Journal of Food Protection* 66: 1184–1189.

United States Department of Agriculture Food Safety and Inspection Service. 2010. *FSIS regulations and policies. Acts & authorizing status*. Available at: http://www.fsis.usda.gov/regulations_&_policies/Acts_&_Authorizing_Statutes/index.asp. Accessed June 9, 2010.

United States Department of Agriculture Food Safety and Inspection Service (2010). *FSIS recalls*. Available at: http://www.fsis.usda.gov/FSIS_Recalls/. Accessed Nov 3, 2010.

Woerner, D.R., J.R. Ransom, J.N. Sofos, et al. 2006. Preharvest processes for microbial control in cattle. *Food Protection Trends* 26: 393–400.

Younts-Dahl, S.M., M.L. Galyean, G.H. Loneragan, et al. 2004. Dietary supplementation with *Lactobacillus*- and *Propionibacterium*-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. *Journal of Food Protection* 67: 889–893.

Younts-Dahl, S.M., G.D. Osborn, M.L. Galyean, et al. 2005. Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. *Journal of Food Protection* 68: 6–10.

Zhao, T., M.P. Doyle, B.G. Harmon, et al. 1998. Reduction of carriage of enterohemorrhagic *Escherichia coli* O157:H7 in cattle by inoculation with probiotic bacteria. *Journal of Clinical Microbiology* 36: 641–647.

Zhao, T., S. Tkalcic, M.P. Doyle, et al. 2003. Pathogenicity of enterohemorrhagic *Escherichia coli* in neonatal claves and evaluation of fecal shedding by treatment with probiotic *Escherichia coli*. *Journal of Food Protection* 66: 924–930.

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