

**DIRECT FED MICROBIALS FOR SWINE:
PROBIOTICS AND COMPETITIVE EXCLUSION CULTURES**

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The administration of beneficial microorganisms as Direct Fed Microbials to swine and other animals to improve gut function, enhance health or to contribute a variety preferred enzymatic activities is conceptually attractive. Traditionally, the term “probiotic” (meaning “for life”) began being used to describe microbial supplements for animal feeds and the following definition was subsequently formalized, “live microbial feed supplement which beneficially affects the host animal by improving intestinal microbial balance”. Probiotic supplements consist of viable microorganisms Generally Recognized as Safe (GRAS) and thus are not usually regulated as drugs. Competitive exclusion cultures were specifically developed as beneficial mixtures of viable microorganisms originating from a healthy host animal and were initially intended as products designed to facilitate the natural colonization process of animals now reared in intensive production environments. Bacteria within competitive exclusion cultures are intended to establish and colonize within the gastrointestinal tract of treated animals and are considered drugs by the U.S. Food and Drug Administration because they affect the form or function of inoculated neonate animals. Consequently, costs of obtaining regulatory approval for true competitive exclusion cultures has been a major deterrent to their commercialization and at present no FDA approved competitive exclusion cultures are available for swine. In practice, probiotics and competitive exclusion cultures are often used for the same purpose, to exclude unwanted bacteria or their activities from the animal gut, and thus the terms are frequently used interchangeably. Results from valid scientific studies with numerous commercially available products have often been variable. Failure to demonstrate irrefutable verification of efficacy has been attributed to the complexity of the gut environment, our lack of understanding of their potential modes of action and the myriad of various application protocols and measured outcomes assessed. With respect to food safety and animal health applications, probiotic and experimental competitive exclusion cultures have been shown to reduce carriage and shedding of zoonotic pathogens in food-producing animals. Probiotic and competitive exclusion cultures have also been shown to reduce mortality and to decrease medication costs in young swine infected with enteropathogenic *Escherichia coli*. Probiotic and competitive exclusion applications to reduce the contribution of swine to environmental emissions such as phosphorus, nitrogen, odors and to enhance manure decomposition are being investigated. As our understanding of the gastrointestinal system and how to manipulate the various populations residing in this environment improves more efficacious probiotic and competitive exclusion technologies are likely to emerge. Additionally, economic and political pressures to reduce antibiotic usage in animals is anticipated to motivate continued research and development of probiotic and competitive exclusion technologies.

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OVERVIEW OF DIRECT FED MICROBIALS

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Direct Fed Microbials (DFM) are one common example of a probiotic approach to improving animal health, performance, efficiency and food safety. In this presentation, the theories behind how probiotic approaches are targeted, general definitions and why they are fed to animals are discussed. Descriptions of DFM, prebiotics and competitive exclusion cultures are provided. Further prospects for introducing new probiotic cultures are described. What are the future prospects of probiotic approaches if antibiotics are removed from the production arsenal? Attendees are challenged to view probiotics as one tool in the arsenal of improving food animal production that can be used synergistically with several simultaneous probiotic approaches.

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CHALLENGES OF DIRECT-FED MICROBIAL APPLICATION TO NEONATAL AND NURSERY PIGS

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The importance of colonization by commensal microbiota for the development of a functional gastrointestinal and immune system has been widely recognized and well documented in the scientific literature. Development of effective direct-fed microbials should capitalize on the succession process by identifying key members of the microbial community associated with desired functions and thereby dictate a portion of the microbiota to benefit health and production of livestock species.

Early administration of direct-fed microbials is crucial to enhance the development of beneficial commensal microbiota and for efficacy during neonatal disease challenges. Work in our laboratory has demonstrated that *Lactobacillus brevis* strain 1E1 must be supplemented to the piglet during the pre-weaning period to improve the growth response during the nursery phase. Milk supplement with *L. brevis* provided to litters in addition to the lactating sow has afforded a means of administration to the nursing pig, although these systems are not often feasible under commercial production conditions. The sow provides an excellent delivery vehicle for direct-fed microbials via fecal-oral transfer of supplemented bacteria to the pig. A *Bacillus*-based direct-fed microbial has been documented to be present in three to five day old piglets nursing supplemented sows and these pigs had lower clostridial counts in the gastrointestinal tract compared to pigs nursing unsupplemented sows.

Stability of live bacteria is often the focus when considering direct-fed microbials in animal feed delivery systems; however matching specific beneficial microorganisms with the appropriate production phase is not often considered. We have observed in our laboratory that candidate direct-fed microbial strains of the same species can elicit very different responses depending upon the stage of production in which they are administered. Specifically, one strain of *Pediococcus acidilactici* improves litter performance when provided during the lactation phase but results in decreased growth performance when provided during the nursery; whereas, a different *P. acidilactici* strain improves nursery pig performance when administered in the nursery phase.

The challenges of supplementing direct-fed microbials to the young pig go beyond considerations of stability of live microorganisms in animal feed. Appropriate strain selection and timing of administration to the pig must be considered to ensure the direct-fed microbial has an opportunity to elicit the desired benefit.

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PREBIOTICS OF PLANT AND MICROBIAL ORIGIN
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The use of ingredients that result in health benefits to, and disease reduction in, the host has gained significant attention in the food and feed industries. Select non-digestible oligosaccharides have been reported to lower risk of infection and diarrhea, improve immune system response, and modulate colonic microbiota concentrations. Prebiotics are ingredients that escape enzymatic digestion but are partially fermented by cecal/colonic microflora and selectively stimulate the growth and (or) activity of bacteria that contribute to beneficial effects on colon and (or) host health. Prebiotics are prepared as a result of direct extraction from plants, controlled hydrolysis of polysaccharides, or enzymatic synthesis by microbes. Fructans, galactooligosaccharides, and lactulose are established prebiotics. Within the fructan family, inulin is extracted by hydrolysis of chicory root and artichoke. Inulin can be further hydrolyzed to oligofructose. Short-chain fructooligosaccharides result from microbial action on sucrose. Galactooligosaccharides result from microbial action on lactose. Lactulose is manufactured by alkaline isomerization of lactose. These have been shown to selectively stimulate growth of bifidobacteria and lactobacilli, while decreasing potentially pathogenic bacteria (i.e., *Clostridium perfringens* and *E. coli*) in both humans and animals. Potential prebiotics include isomaltooligosaccharides, xylooligosaccharides, and lactosucrose, but there is not yet sufficient evidence for them to be termed prebiotics. Research results are promising, however. Isomaltooligosaccharides are manufactured in a two-stage process: first, starch is hydrolyzed to soluble starch. Second, β -amylase hydrolyzes starch to maltose, which then is transglycosylated to isomaltooligosaccharide. Xylooligosaccharides are found naturally in several plant sources including corn cobs, straws, and hardwoods, and are enzymatically extracted from the raw materials. Lactosucrose is manufactured by the transglycosylation of lactose and sucrose with the enzyme, β -fructofuranosidase. Furthermore, many other carbohydrates and ingredients are being investigated for their ability to act as prebiotics (e.g., soybean oligosaccharides, glucooligosaccharides, resistant starch, whole grains). Non-carbohydrate compounds with possible prebiotic effects include lactoferrin, phenolic compounds, and glutamine; however, there is insufficient research to date to define them as prebiotics. While there are limited compounds available with proven prebiotic effects, many novel compounds are currently being investigated, and the list of ingredients with prebiotic activity undoubtedly will continue to grow.

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The Challenge of Delivery of Microbials to Ruminants

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The delivery of microbials to ruminants has numerous steps and presents various technical obstacles. These steps include: fermentation, harvesting, packaging, storage, transportation to the farm, then application to animal feed that is delivered to the animal for consumption.

In commercial settings, DFM application methods to animal feed have had a tremendous impact upon bacterial viability. The most challenging steps of delivery involve exposure of a live microbial to the wide range of environmental conditions until reaching the rumen. These environmental conditions include: atmospheric temperature, atmospheric humidity, feed temperature (e.g. steam flaked or rolled corn), milk replacer temperature, feed dry matter content, feed micro-osmolarity, antibiotic concentrations, and water quality.

Specific to ruminants, the two most common methods of DFM application are: 1) dry application (placement of dry powder onto feed) and 2) wet application (dissolving of a dry powder into a liquid that is subsequently sprayed onto the feed).

The wet application method in commercial settings requires technically complex equipment, routine service and maintenance, and dedicated micronutrient application rooms. Because of the management and cost overhead associated with wet application, only larger ruminant operations (e.g. generally dairies over 5,000 head and feedlots over 20,000 head) utilize wet application. Alternatively, dry application methods are used by a greater number of operations. Dry application has proven to be a flexible and robust means of application that is no way inferior to the wet application method. However, because dry application is less automated, there is more room for associated user error.

In most cases, environmental factors can be addressed through site-specific application modifications using wet or dry application methods. Thus, it is acknowledged that customizable DFM technology application is more difficult to uniformly administer across the industries than ionophores, antibiotics, beta-agonists, and hormones. Despite these challenges, DFM use in cattle has generally become an accepted practice in the last few years in large part due to research that has shown, when properly delivered, DFM have improved ruminant production performance and reduced the presence of *E. coli* 0157:H7 in cattle feces. The most widely utilized live bacterial DFM in ruminants today are lyophilized *Lactobacillus* and/or *Propionibacterium*. These non-spore forming organisms pose specific challenges for application because they are not as thermostable as spores and are susceptible to the mentioned environmental factors. Unfortunately, spore-based DFM have yet to demonstrate beneficial results in ruminant applications and their ability to germinate ("become alive") in the ruminant GI system has yet to be established.

Correspondingly, the largest challenge of microbial delivery is that the amount of viable microorganisms delivered to the animal is often disregarded or improperly calculated. This should be underscored by the fact that non-viable microorganisms may have little impact if the DFM modes of action are: epithelial site attachment, altered intestinal fermentation profile, production of metabolites, or bacterial antagonism. In addition, it is well documented in the literature, and from our own *in vivo* and *in vitro* studies, that DFM efficacy can be dose dependant. Thus, sufficient viable microorganisms numbers must be delivered to elicit beneficial effects upon the host.

As a solution to monitoring and improve delivery of microbials in the future, we suggest that diagnostic determination of live bacteria reaching the ruminant must be implemented at the research level and industry-wide. We feel the use of a microbial tool that allows for rapid strain-specific enumeration of target DFM populations is critical to: i) illuminate the modes of actions of DFM, ii) elucidate the proper microbial dosage specific to each type of ruminant, iii) determine the impact of application methods upon viability, iv) assess the effect that environmental factors have upon viable cell populations, and ultimately v) perform accurate and precise application of microbials to feedstuffs of ruminants in commercial settings.

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“OVERVIEW OF DIRECT FED MICROBIALS AND PREBIOTICS: IMPORTANT CHARACTERISTICS AND SELECTION”

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The use of direct fed microbials can have potential beneficial impacts on animals in the form of improved growth and health. They also can have benefits on the microbial safety of meat or milk from livestock. In order to realize these potential benefits it is necessary to select the proper microorganism or mixture of microorganisms for use in the direct fed microbial. There are a lot of organisms that have been promoted as having potential of producing desirable benefits. It is not likely that any one of these organisms can provide all the potential benefits. Thus, it is important to carefully select the organism(s) to be included.

The beneficial effect may or may not require that the organism grow in the intestinal tract. If growth there is required, the organism must be bile tolerant. On the other hand if the function of the organism is to provide a specific enzymatic action the importance of bile resistance is minimal. However, the organism must have the active enzyme at the time it is fed to the animal. Thus growth conditions for producing the direct fed microbial may be critical.

Evidence suggests that some of these organisms exhibit host specificity. Thus source of the organism becomes important. For example, I prefer to use a culture from the intestinal tract of cattle for use as a direct fed microbial for cattle.

The microbial must be one that can be produced easily in a commercial culture supply company. It should have no complicated growth requirements. Furthermore the culture(s) must be stable during storage and in the feed until consumed.

The most difficult thing for which to select is that the direct fed microbial have the desired effect in the animal. Sometimes this can require large feeding trials involving an number of different cultures in order to select the best one(s). If more than one organism is desirable, then they must be compatible. It is desirable that there be laboratory tests to screen the candidate cultures to select the best one(s).

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Direct-fed Microbials: Species Involved

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Currently there are a wide variety of species involved in direct-fed microbial supplementation for animals. The following is a basic statement for including direct-fed microbials in a product: "Contains a source of live (viable), naturally occurring microorganisms." This statement was developed by the industry, FDA, and the Association of American Feed Control Officials. The microbial species deemed suitable for use in animal feeds by the FDA can be found in the Official Publication of the Association of American Feed Control Officials.

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Current State of Pre- and Probiotic Use in Companion Animal Nutrition

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Probiotics are commonly used as nutritional supplements throughout the world. They have been widely used by humans and in livestock for many years however, their use in companion animals, namely the dog and cat, has been much less with a great deal of information appearing in the last few years. Reasons for the slow adoption have been numerous. Applications in livestock relate to growth promotion and as a replacement for antibiotics in the diet, issues not of great importance for companion animals. Applications for humans have been more akin to companion animals seeing use as an aid in the treatment of gastrointestinal disorders. Modifying the gastrointestinal environment has been the goal of much of the research in companion animals where studies seek to demonstrate efficacy through decreased presence of pathogens and increased numbers of favorable organisms in the feces, improvement in diarrhea, or more rapid recovery from diarrhea. Because of demonstrated benefits probiotics have become more commonplace in the treatment of these disorders and as an aid in the prevention of these disorders associated with stress or some other illness.

There now have been numerous studies seeking to select and characterize specific organisms to use as probiotics in the dog and cat. Companion animal foods also present some special challenges for the ready adoption of probiotics into the diet. Canned foods require sterilization which is obviously detrimental to the inclusion of a live organism. Dry foods are also extensively processed by extrusion which makes the prior inclusion impossible however, organisms can be added prior to packaging.

Because of these manufacturing concerns prebiotics have received considerable attention as they can be more readily adopted into current diets. Prebiotic fibers, particularly fructooligosaccharides, have been shown to reduce the numbers of undesirable organisms in the feces, and improve the recovery from gastrointestinal disorders.

The companion animal also offers some unique opportunities for the application of pre- and probiotics with consumer driven issues like fecal quality. Fecal quality refers to the characteristics of the feces that make it easy to collect, like consistency, moisture content, and form. This can also be extended to fecal odor, flatulence and fecal quantity. Anything nutritionally that can improve these qualities is favorable to the consumer. Both pre and probiotic feeding has been shown to have a role in these areas by improving fecal quality, and reducing odor compounds. Several products containing both pre- and probiotics are now available for both the dog and cat.

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DFM (PrimaLac[®]) Modulates Immune System Energy Consumption

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Direct fed microbials (DFMs) are used in food animal production as an alternative to subtherapeutic use of antibiotics to promote animal health. Previous studies have suggested that DFMs improve animal health by several different, integrated mechanisms. These include the prevention of colonization of pathogenic and opportunistic organisms, enhancement of nutrient digestion and absorption, and increased immune function. Our research group is using a systems biology, multi-disciplinary, approach to describe how DFMs may aid the host in resisting pathogenic challenges. We have employed the broiler chick supplemented with the DFM consortium, PrimaLac[®], as our model. We have estimated DFM mediated changes to host energy consumption; both whole-animal and organ, as well as innate and adaptive immune function. The results of our studies suggest that DFM treatment in healthy broilers results in the repartitioning of energy amongst broiler organs, but does not cause increases in whole-body energy expenditures. This increase in energy consumed by the tissues and cells of the immune system explain, in part, how DFM treatments result in enhanced immune responses to pathogenic challenges. Our data underscore both the complexity of the DFM/broiler interaction, and the need for a greater understanding of how intestinal physiology and immunity are interrelated.

Key words: direct fed microbial, broiler, energy metabolism, immune function

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Animal Species Applications – Direct Fed Microbials and Prebiotics – Beef Cattle

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Direct-fed microbial (DFM) products are fed to improve performance of feedlot cattle. Research data shows benefits in rate and efficiency of growth. Favorable results of including DFM in diets of feedlot cattle were first reported in 1988, with similar reports appearing regularly since then. Studies conducted through 2002 were summarized for bacterial species of *Lactobacillus* or *Propionibacterium* fed separately or in combination at levels ranging from 10^5 to 10^9 colony forming units (CFU) per head per day. Results suggested that feeding bacterial DFM to feedlot cattle resulted in a 2.5 to 5% increase in daily gain and an approximately 2% improvement in feed efficiency, whereas changes in dry matter intake were not consistent. In studies reviewed, carcass weight was numerically increased, but no other differences in carcass merit were observed. Studies conducted since the 2002 review suggest very similar results. Experiments conducted at universities and in large-pen commercial feedlots show that feeding bacterial DFM in diets of finishing cattle increases daily gain 2.0 to 2.5% and improves feed efficiency approximately 2%, similar to the 2002 data summary. These average results would produce economic benefits for feedlot cattle producers. Assuming increased feed efficiency results from increased gain, the value of improved feed efficiency in finishing diets increases as ration costs increase, making DFM potentially profitable with increasing feed costs. However, in order for the addition of a DFM product to be profitable the cost:benefit ratio must be low.

There is also research evidence that suggests DFM have favorable effects on health of feedlot cattle. Studies conducted through 2002 suggest added performance and decreased morbidity due to DFM in the receiving period (average length of 30 days). Favorable health effects of DFM may be situation-dependent, as positive results were not always observed. It has been speculated that extremely sick or extremely healthy calves might be less likely to show a health-related response to DFM. However, improved health when feeding a DFM could manifest itself as reduced death loss or increased performance in calves at high risk of stress and/or disease. In the feedyard, data suggest that DFM can improve growth performance, and performance improvement may be greater in high-risk cattle. In addition, mortality is reduced when a DFM is fed. However, current data does not describe the efficacy of individual DFM products under controlled conditions.

Although feedlot cattle are not currently fed for the sole purpose of enhancing pre-harvest food safety, strains of *Lactobacillus acidophilus* have been shown to decrease fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle at harvest. Taken together, the published data indicate that DFM can improve health and performance of feedlot cattle. Data provide support for use of DFM in feedyards, and help to explain why approximately 35% of U.S. feedlot cattle receive a DFM product.

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Microbials, Prebiotics, Acids, Other Feed Additives, Symbiotics in Aquaculture

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The total farm gate value of aquaculture is over \$20,000,000,000. The farm gate value for marine shrimp alone is over \$10,000,000,000 and is increasing at a rate of over 8% per year. Aquaculture production technology has greatly intensified during the last two decades. For example, 1,000 to 2,000 pounds of shrimp were produced per acre per year without the use of supplemental feed during the 1980's to today with the production increasing to between 300,000 to 500,000 pounds of shrimp per acre per year. With the maturing of the aquaculture industry with intensification, the reduction in production due to disease and stress has become a major concern. Some areas of the world are experiencing over 50% loss in shrimp production due to disease. This has also led to the use of antibiotics (e.g. oxytetracycline) in aquaculture feeds to reduce the effect of disease on production. This indiscriminate use of antibiotics in aquatic production systems has resulted in documented development of disease resistant strains of *Vibrio*, a major bacterial pathogen in shrimp production. It is critical that prebiotics, probiotics, acids, and/or immunostimulants be used instead of antibiotics in aquatic production systems. In this respect, prebiotics and/or acids have been reported to increase shrimp production over 10% in Ecuador, the leading country in the western hemisphere for the production of shrimp.

Both agriculture and aquaculture use animal husbandry under control conditions for the production of food for human consumption. The basic concept for the farming of terrestrial and aquatic animals is similar. In fact, many of the basic features of the swine and chicken industry have been used as guidelines for the intensification of the shrimp farming industry. However, there are major differences. Just considering water versus air major differences exist in terms of the efficacy of feed additives. For example, in water the stability of the feed and associated leaching of the feed additives into the water and the major difference in technologies for the production of aquatic feeds may be a major problem in terms of delivery of the feed additive to a slow eating aquatic animal. There are over 20 species of aquatic animals in four major phyla, mollusca, arthropoda, echinodermata, chordata, representing major differences in life and production strategies which are commercially farmed today. There are major differences between the farmed aquatic species in gut passage times, gut flora, feed consumption rates, immune systems, digestive systems, nutrient requirements, etc. The lack of basic information concerning the gut flora, immune systems, digestive systems, etc. of aquatic invertebrates present a major problem in determining the mode of action and how the prebiotics, immunostimulants, etc. should be used.

Probiotics have been added directly to the pond water and bottom to increase their quality. Though it is a fact that the addition of probiotics to pond water and soil can affect the flora, water and soil quality it is also very expensive. The addition of probiotics to feeds has been successful for some species (e.g. fish). However, in other species such as shrimp with their short gut passage time of less than 60 minutes the effect of probiotics in the feed is questionable. Acids are being added to feeds used for pond production of shrimp and it is estimated that there is a resulting 10% increase in production. Prebiotics are also being used in feeds for commercial production of shrimp with success. Also, laboratory research has shown that prebiotics increase growth, change the gut flora and affect the immune parameters of shrimp. There is an urgent need to replace the indiscriminate use of antibiotics in aquatic feeds. The use of microbials, prebiotics and immunostimulants either singly or in combination offer a solution for the replacement of antibiotics in aquatic feeds with resulting increase in production. However, the information concerning dosage levels, when the feed additives should be used, the conditions under which they should be used, etc. is not adequate for most feed additives. The major restraint for the use of probiotics, prebiotics and immunostimulants in aquatic production systems is the cost versus a predictable increase in the internal rate of return. Finally, it is suggested that aquatic species, e.g. zebra fish, sea urchin and starfish, can be used as models to determine the mode of action, efficacy, etc. for microbials, prebiotics and immunostimulants in all animals.

Function of Normal Intestinal Microbiota in Animal Health

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The mammalian and avian intestinal tract contains up to 10^{10} bacterial cells per gram of contents consisting of between 400 and 1,000 different bacterial species. The majority of the bacteria are beneficial, with a few that may either be opportunistic pathogens that normally colonize the intestinal tract in low numbers, or may be transients. These microorganisms are in dynamic communication with the host epithelium, mucosal immune system and hypothalamic-pituitary-adrenal axis. Animal health and productivity depends upon the interplay between these systems. It is unclear at present whether the ability of the commensal microbiota to inhibit pathogens is due to specific bacterial species, or to the diversity of bacterial species and metabolic activities that are present. The intestinal tract is a multitude of competitive dynamic ecosystems. The microbial composition is thought to be stable at higher levels of relatedness, suggesting that general characteristics may be stable. However, the situation is much more dynamic at the species and strain level. Direct fed microbials, prebiotics, synbiotics and postbiotics have to function not only in a complex microbial environment, but also with complex inputs from the epithelium and the mucosal immune system, which are all influenced by hormonal and endocrine inputs. Consideration of the complexity of the systems is critical for developing effective dietary additives that improve animal health and performance.

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CURRENT STATUS AND FUTURE PROSPECTS OF DIRECT FED MICROBIALS AND PREBIOTICS

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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.

MECHANISMS OF ACTION OF DIRECT-FED MICROBIALS

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The cattle industry is continually searching for alternatives to improve animal performance, animal well-being and profitability. A recent technology that has been adopted by a portion of the cattle feeding industry is the oral use of selected bacteria (direct-fed microbial; DFM) to improve growth performance and reduce shedding of *E. coli* O157:H7 in feces. A summary of the performance response from feeding a DFM is shown in Table 1. The summary is from 73,870 lots that contained 10,900,504 head of cattle. Given the size and robustness of the database, the reported advantage is very likely to be repeatable and consistent throughout the industry. This cited performance advantage equates into \$5-10/head gross income when cost of gain is \$80/cwt. Fecal shedding of *E. coli* O157:H7 has been reduced by 30-60 % in several reports when a DFM is fed. The response appears to be consistent among the DFM tested. The reduced fecal shedding of *E. coli* O157:H7 is a further advantage that has less of a direct monetary benefit. In the future, the food safety benefit may become more important than the performance response.

Table 1. Performance response of cattle to DFM

	Steers	Heifers
	Advantage over cattle w/o DFM, %	
ADG	1.9	1.4
Feed efficiency	1.9	3.4

McDonald et.al. 2005

One of the challenges the cattle industry struggles with is acute acidosis (accumulation of lactic acid in the rumen) which can significantly lower performance and well-being of dairy cows and feedlot steers. One potential remedy is to feed a DFM in the diet to metabolize the lactic acid. Several DFM's have been evaluated but the response has been variable. Strategies need to be implemented to obtain a more consistent and prolonged response to a DFM to minimize the frequency of acidosis events. More research is needed in this area. The search will continue because of the magnitude of losses suffered with acidosis.

Another area that has received research attention is the influence of DFM on intestinal immune function. DFM have been shown to increase intestinal phagocytosis, natural killer cell activity and immunoglobulin when fed to livestock. *Lactobacillus* has been shown to bind to intestinal epithelium and block colonization by undesirable organisms in multiple species. DFM's have been shown to enhance recovery of the intestinal ecosystem after a scouring/diarrhea event. Potentially, DFM may be a natural remedy to replace or augment antibiotics. The acute phase proteins secreted by the liver in response to bacterial invasion in the blood have been stimulated when a DFM was fed to the cattle. The impact of DFM on immunity is an exciting and growing area of research.

Successful application of this technology to the livestock industry will improve health, animal well-being and food safety. It has the potential to reduce antibiotic use in livestock.

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Challenges of delivery of microbials to animals: Aquaculture

Wendy Sealey

The level of interaction between the intestinal microbiota of aquatic animals and their surrounding environment is substantially greater than that of terrestrial animals. This interaction is due, in part, to the fact that eggs and larvae are released into the environment at early ontogenetic stages. From this point, aquatic animals are continually exposed to environmental microbes through the flow of water and food through the gastrointestinal tract. Not surprising, interactions are greatest for those aquatic species that filter feed and for larval fish which lack gastric barriers. In contrast to terrestrial animals, gram-negative facultative anaerobes are predominant with genera dependent on salinity and temperature in these poikilothermic animals. Due to the complex and fluid nature of these interactions most aquatic species gastrointestinal microbial communities are somewhat transient and generally believed to primarily reflect the aquatic environment and feedstuffs. Thus in aquaculture, direct microbial delivery methods include addition to the water, enrichment of larval foods, and inclusion in the diet.

Challenges to direct microbial delivery in aquatic species include the vast quantities necessary for water addition, diversity of aquaculture species and culture methods employed and the lack of knowledge of microbial ecology of intestinal tract and culture environment. Instability of microbials during processing, feed production and storage also limit direct delivery in prepared feeds. Up to this point, these challenges while acknowledged, have only received minimal research attention. Instead, the majority of microbials research in aquatic animals has been laboratory-based and focused on bioremediation and reducing disease outbreaks.

Currently, an increasing number of novel and re-purposed dietary supplements are becoming commercially available for more far ranging aquaculture applications. As an example, probiotic bacteria and yeast products, which have previously been primarily investigated for their anti-pathogenic effects in fish, are now promoted to aid in plant-based diet utilization and digestive tract health. These products along with prebiotics, essential oils and organic acids, which also modify microbial communities and are more amenable to aquafeed production, show promise in research settings as direct fed microbials for both novel and traditional disease-related prevention in aquatic species. However, additional “real world” research is needed to adequately substantiate label claims and improve consumer confidence for the various products.

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The regulatory role of direct fed microbials on pig immune response and gut function Gloria Solano-Aguilar¹, Terez Shea-Donohue², Marta Restrepo¹, Harry Dawson¹, Joseph Jr. Urban¹. ¹Diet Genomics and Immunology Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture. ²Mucosal Biology Research Center, University of Maryland, School of Medicine.

The gastrointestinal tract contains a complex commensal microbiota that contributes to the homeostasis in the gastrointestinal tract. This balance is often disturbed by disease onset or dietary interventions. Probiotics are live microorganisms that when administered in sufficient amounts provide some positive health benefit; strains of *Lactobacilli*, *Bifidobacteria* and *Enterococcus* are the most commonly used probiotics. Experimental data with controlled colonization of probiotic *Lactobacilli* and *Bifidobacterium* strains in baby-pigs as an animal model for humans can now be accomplished immediately after birth by direct oral treatment or through feeding the gestating mother setting up the conditions for a higher colonization. Functionality of probiotics has been tested individually for specific applications since age, host site, probiotic vehicle and dose, type of diet, length of probiotic exposure and environment are factors that influence the establishment, maintenance and effect of the probiotic strain in the host. The type of association of the probiotic strain with the host's mucosa needs to be carefully characterized before beneficial claims are proposed. The effect of probiotics under disease conditions has also been tested. A prototype allergic response induced by food proteins or parasite infection has been used to test the modulatory effect of certain strains when there is a detrimental effect on intestinal epithelial function (determined by reduction in Transepithelial Electrical resistance (TEER) or by alteration in nutrient absorption. Human derived probiotics can modulate immune function and selectively affect local responses to parasite infections or dietary changes while promoting swine health. This biomedical model can be extended to assess the activity of selected probiotics on pig responses to other infectious agents that negatively affect pig production.

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DIRECT FED MICROBIALS IN DAIRY CATTLE

Doug Ware, Ph.D.

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The dairy industry plays a vital role in the US and global economies. In 2004, farm cash receipts from cow-derived milk and cream sales were over \$27 billion, accounting for over 10% of all US farm receipts; (IDFA, 2005) however, the increased cost of production has contributed to a 23% reduction in the number of dairy operations functioning in the US since 2001 (USDA, 2007). To improve the profitability and sustainability of dairy farming, new technologies that positively benefit the farmer and are perceived positively by the public need to be evaluated.

One such technology that is increasingly being incorporated into dairy operations is the administration of probiotics, or more properly referred to as direct-fed microbials (DFM), into dairy rations. The increased adoption of DFMs is a clear indicator of the farmer's experienced and perceived benefit. Based upon NPC and DFM industry sales data, the last 15 years has seen a steady increase in the direct application of beneficial bacteria to feedstuffs in all ruminants including dairy cattle.

Exploring why the increased use of DFMs has been so rapid, we conducted a literature review of published peer reviewed studies. Unfortunately, for DFM studies, statistically significant data is difficult to cite. We hypothesize that in part limited research budgets and small of animal numbers ($n = \#$) have contributed significantly to limiting the adoption process from becoming even more significant. With the large biological variability of daily production and limited cow numbers employed in these studies, it is difficult to observe statistically different responses when applying DFM in scientific settings. Additional observations from DFM studies across multiple domestic animal species (beef, swine, and poultry) suggest that direct-fed microbials demonstrate greater potential in lower-performing animal rearing facilities than those with near optimum animal performance. Because most research trial take place in optimal settings this might explain some of the lack of, or minimal benefits, often seen in organized research trials as that are in conflict adoption rates of DFM's by producers and field/farm observations of production dairy herds.

To explore why the adoption rate of DFM's in dairy cattle has steadily increased at such a dramatic rate, we review in this analysis studies that report "trends" in data that support improved animal performance and production by the feeding of DFM's that include Yeast, *Enterococcus*, *Propionibacterium*, and *Lactobacillus*.

Promising studies evaluating the addition of DFM include: a) feeding yeast to dairy cows may enhance cellulolytic activity and increase milk production in certain stages of lactation, b) feeding of *Propionibacterium* increases in rumen propionate concentrations and positively affects blood glucose and insulin concentrations, c) feeding of *Lactobacillus* and *Propionibacterium* may increase daily milk yields and improve feeding efficiencies. Lastly we review the idea of herd health in dairy cattle as important reason for adoption of a DFM strategy due to the statistically significant observations that d) the administration of *L. acidophilus* and *P. freundenreichii* to cattle feed reduced detection of *E. coli* O157:H7 in feces from 27% in control animals to 13% ($P = <0.05$) in treated animals and hide contamination was significantly reduced from 13.3% to 1.6% ($P = <0.04$) and e) the feeding of *Lactobacillus acidophilus* can positively influence intestinal physiology and intestinal morphology ($P = <0.01$).

The research horizon for the application of direct fed microbials offers significant opportunities to improve the production, health and welfare of dairy cattle while concurrently reducing the incidence of animal and food-borne pathogens associated with dairy production.

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What are Direct-fed Microbials?

Steven Woskow, Ph.D.

In the U.S.A., the official definition (Association for American Feed Control Officials) of Direct-fed Microbials (DFM) is “a source of live (viable) naturally occurring microorganisms”. The use of live bacteria to promote health was first proposed at the turn of the century by Dr Eli Metchnikoff. Dr. Metchnikoff promoted the theory that aging was caused by toxic bacteria in the gut and ingestion of lactic acid bacteria could prolong life. He based this on population studies in Eastern European countries that consumed large quantities of fermented milk and had extraordinary life spans. He believed consumption of fermented milk would “seed” the intestines with lactic acid bacteria that would suppress the growth of putrefactive proteolytic bacteria. Indeed, the use of DFM today are largely based on the belief that lactic acid bacteria populate the intestinal tract and promote good health by excluding bacteria that cause disease. However, this is a very static view where the gut bacteria play a passive role in the health of the animal. Yet, this view has been the basis for the use of DFM in man and animals since Metchnikoff's time. In the past, our knowledge of the gastrointestinal tract and the bacteria that inhabit it was largely based on traditional microbial culturing techniques. While traditional microbial enumeration, selection and isolation techniques are important, the information acquired is limited. Like all biological sciences, the application of the polymerase chain reaction (PCR) has changed the way microbiologists conduct research and transformed our understanding of the role of the gut microbiota. Now microbiologists have a variety of cultivation-independent techniques to study the bacterial diversity in the gastrointestinal tract both spatially and over time. These new molecular based methodologies have allowed scientists to gain insight into pathogens associated with disease, identify DFM candidates associated with healthy high-performing animals and monitor microbial succession during specific phases of production. For example, microbial diversity can be determined by comparative analysis of bacterial 16S ribosomal genes using techniques such as TFRFP (terminal restriction fragment length polymorphisms) and DDGE (denaturing gradient gel electrophoresis). These techniques allow scientists to understand microbial diversity over an entire population rather than just a few predominate microorganisms. By using a real-time PCR-based method such as Suppression Subtractive Hybridization (SSH), scientists can separate two closely related DNA samples by excluding sequences that are common to the populations. These unique DNA fragments can be sequenced, cloned and used as probes to identify unique novel DFM candidates. SSH also allows scientists to gain insight into the functional capabilities of microbial communities under different environmental conditions. These new molecular techniques are changing how we view the gut microbiota – they are no longer thought of as passive inhabitants of the gastrointestinal tract, but play a dynamic and active role in the health and well being of man and animals. In the future, scientists will gain deeper insight into microbial diversity of the gastrointestinal tract, the functional role of specific species within the bacterial population and how they interact with the host organism. The knowledge gained will help us better answer the question – What are Direct-fed Microbials?