

Strategies to reduce the shedding of *Escherichia coli* (STEC) O157:H7 and non-O157:H7 using probiotics strains into animals

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Shiga toxin-producing *Escherichia coli* (STEC) strains are food-borne pathogens that cause human diseases, and ruminants are usually important reservoirs of STEC. The most notorious of the STEC is serogroup O157:H7, which represents a major public health concern worldwide. Yet in some countries, non-O157 STEC strains are more frequently isolated than O157 strains from meat-producing animals and as contaminants in foods. Transmission of these pathogens can be from person to person, or via contaminated water, although the primary transmission pathway is considered to be the consumption of meat products or other foods contaminated, during or post slaughter, with animal fecal material. The first step of enteric infection is colonization of the host's gut mucosal surface by pathogenic strains of bacteria. Afterward the pathogens are able to cause infections causing changes in both physiology and morphology of the host.

It is clear that on-farm management practices especially the maintenance of pathogen free feed and water may be the most practical means of reducing the incidence infectious agents in production animals. In addition a number of pre-slaughter on-farm control methods, have been proposed or are currently used in some countries to manipulate the microbiota of the rumen and/or intestine as a means of reducing or eliminating the shedding of STEC by ruminants. Such approaches may in turn serve to reduce contamination of hides. Reported methods include, switching from a high grain to a high forage diet prior to slaughter of cattle, the inclusion of essential oils and citrus products in feed, the and the feeding of viable microorganisms, commonly referred to as probiotics, but more correctly defined as direct-fed microbials (DFM) according to the Food and Drug Administration (FDA) of the United States of America. The purpose of this chapter is to present several strategies that have been used to reduce the shedding of *Escherichia coli* O157:H7 and non-O157:H7 on the farm using probiotic strains, thereby reducing transmission to humans. The aim is also to show the mechanisms of action and the parameters that affect the complex relation pathogens - probiotics strains. The rumen compartment as being a relevant target for reduction of the number of viable STEC cells, such intervention strategy may be the use of probiotics supplemented in the rations; however, it is not the only way, which could be used with this finality. The present chapter will try to approach the importance of microbiota to impart a health benefit, its relation with the host immune system, restoring microbial health to the intestinal ecosystem, probiotics and its mechanism of action. Some studies have purposed that any benefit on the gut's health would lead to a reduction of the shedding of pathogens and thus decrease these pathogens under practical conditions. The use of probiotics with this object has demonstrated relevant findings.

Keywords probiotics, *Escherichia coli*, Shiga-like-toxin, non-O157:H7

1. Introduction

The specific mechanisms responsible for increased or decreased *E. coli* O157 and non-O157 shedding or survival are unknown but are often attributed to changes in hindgut ecology induced by diet types. Some interventions may have a direct bacterial effect. Some studies have shown results conflicting or not repeatable, demonstrating the complexity of the hindgut ecosystem. The hindgut ecosystem is affected by feed utilization and variation within feed product. In this context, the variations and alterations of the naturally occurring microorganisms in the gut of the animals may result in various consequences for the animals. One of them is the colonization and establishment of the pathogenic bacteria such as STEC.

This chapter will discuss some strategies, which may be used to minimize this colonization and consequently the shedding by animal's fecal.

All animals that were born under sterile conditions have their first contact with microorganisms through their mom or through the environment. The mother's vagina provides great account of microorganisms and they will be the first microbial population of the skin, mouth and intestinal tract of their progeny.

The normal microbiota has important function to prevent infections and diseases caused by different microorganisms. For appearance of diseases, first of all, a sequence of determined factors are needed of such as; adherence, resistance against the defense of the host; getting of nutrients; colonization and population growth. At this moment the competition, held by microbiota between the microorganisms is intense including the synthesis of several antimicrobials agents resulting few accounts of microorganisms from diverse species.

The incidence of diseases is influenced by the balance of the gut microbiota, so that the constituents, such as bacteria, viruses and eukaryotes interact with one another and also with the immune system of the host.

Microbes present in the gut have an important role in the well-being of their host and some studies have suggested an intensive interaction between the bacteria and the host immune system in way that could lead to development of

diseases. The microbiota consists of bacteria, viruses and eukaryotes that have a great impact on human health. That is why, frequently, it is called the “forgotten organ” and it has been involved in interactions with immune system, providing signals to promote the maturation of immune cells and collaborating to normal immune functions. It is also involved in energy harvest and storage as well as in several metabolic functions such as fermenting and absorbing undigested carbohydrates. Probably this interaction between microbiota’s constitutes is a consequence of the strong evolutionary force that has established these bacteria as humans symbionts.

Some studies have demonstrated a large variability in the composition of the community in healthy individuals with twins sharing less than 50% of their species level bacterial taxa and fewer viral sequences.

Healthy adult’s individuals have a relatively stable of composition of bacteria, viruses and eukaryotes over time. On the other hand, this temporal condition assumes that several parameters such as diet, diseases and environment are kept constant.

In recent years divers methods of detection, identification, and molecular characterization of O157:H7 STEC have led to a more accurate assessment of the role of this serotype in human disease outbreaks and the transmission of infections from animal reservoirs. A major challenge will now be to get a better understanding of how these bacteria colonize the gut of the animal host. Such insight will permit the development of effective strategies to reduce the number of the bacteria in the animal reservoir, and their shedding on the farm. One of these strategies could be the use of probiotics supplemented into their compound feed ration, which many studies have demonstrated that the more effective reduction of *E. coli* O157:H7 and non-O157 STEC shedding can be obtained by treatment with multiple probiotic bacteria.

2. Probiotics

Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts. In the last fifteen years the use of probiotics strains has been shown as able to modulate the balance and activities of the gastrointestinal microbiota in which are responsible to different proprieties on the gut as mentioned above. To modulate the gastrointestinal tract (GIT) of domestic animals providing to them the possibility to maintain the homeostasis of these complex microbial communities is essential, firstly the understanding of the digestive ecosystems in terms of microbial composition and functional diversity.

The reduction of microbial in GIT can be caused by different factors such as feeding practices, composition of animal’s diets, productivity and farms management. The consequence of this state is that, some reactions as digestion and fermentation of plant polymers are impaired, since the realization of these reactions are highly related with the action of the microbiota on the gut and these actions have a fundamental role to the herbivorous.

The EU legislative framework has regulated all additives in animal feed. Before that, until May 2003, all risk assessment of animal feed additives in Europe was regulated by Scientific Committee of Animal Nutrition (SCAN). After this date, overall the functions of SCAN have been taken by European Food Safety Authority (EFSA). While EFSA provide experts scientific advice to the European Commission to approval and to verify the risk management of a probiotic product have been responsibility of the EC and its constituent member’s state as well as in United States for use of microorganisms as a feed additive is necessary before, that the product to be outgoing to approval by Food and Drug Administration (FDA).

The identification and characterization to species levels, and the efficacy data are some requirements for novel probiotic product required by EU regulations. Moreover, some characteristics are requested to product such as no adverse effects on the health of performance the product must be safe for the operator, have no adverse effects upon exposure and also the product not pose a risk to the safety of the end-consumer.

The intensive production farmed livestock together with the veto of the use of antimicrobial feed supplements in the EU, this situation has increased the risk of contracting gastrointestinal diseases if prophylactic antimicrobial feed supplements are not utilized. The removal of growth promoters has led to a significant increase in the incidence of diseases and consequently a significant increase in feed costs with both the reduced feed weight conversion and loss of animals.

3. Mechanisms of action

Probiotics have been the topic of many studies over the past 20 years. Metchnikoff and Tissier were the first to make scientific suggestions concerning the probiotic use of bacteria. They suggested that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora. In the last 20 years many research in the probiotic fields have progressed considerably and significant advances have been made in selection and characterization of specific probiotic cultures. Probiotic bacteria are a subset of specific microorganism, which when ingested, transiently provides to gastrointestinal tract health benefits. Lactic-acid producing bacteria (LAB), particularly members of the genus *Lactobacilli*, *Bifidobacteria*, nonpathogenic Gram-positive bacteria and some yeasts such as *Sacharomyces boullardii* have been used as probiotic agents. The host defense mechanisms can be enhanced by specific probiotic bacteria. The

mode of action of probiotics regarding the prevention of fecal shedding of *Escherichia coli* could be to give supplementary support to the body's naturally occurring gut microbiota, by affecting mucosal immune mechanisms and by stimulating non immune mechanisms through competition/antagonism with potential pathogens. Once the probiotic bacteria reach the intestine, they start to proliferate, metabolize food and produce lactic acid. The local pH is lowered and thus a disadvantageous milieu for pathogens, such as *Escherichia coli* (STEC) O-157:H7, is created. On the other hand, probiotics produce bacteriocins, which decrease the population of potentially pathogenic microorganisms other possible beneficial effects through probiotics for the animals are:

- Suppression of putrefactive processes in the intestine and restoration of the intestinal microflora;
- Some species (e.g. *Lactobacillus bulgaricus*) show clear antimicrobial activity against *E.coli*;
- Drop of mortality;
- Stimulation of the barrier function of the intestinal pathogens;
- Competition for adhesion to intestinal and pathogenic microorganisms;
- Activation of the synthesis of proteins, such as interferon (*Streptococcus thermophilus*) and thus activation of the protective functions of the organism of the animals;
- Reduction of changes in DNA, caused by the action of chemical mutagens in the intestines.

The mode of action of prebiotic is a bit different from that of probiotic. The prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating the growth or activity of a limited number of bacterial species in the colon.

The oligosaccharides are considered the main units between prebiotics, which includes fructooligosaccharides (FOS), inulin, lactulose and galactooligosaccharides (GOS). Synbiotics are a combination of probiotics and prebiotics and it is the synergy between these two substances that is known as synbiotics.

Intestinal bacteria have a major effect on enhancing secretory immune function. Among the more consistently found effects of species Bifidobacteria and Lactobacillus in neonate population is the effect on humoral immunity, particularly on secretory IgA and other immunoglobulins. Bifidobacteria and Lactobacillus given orally to animals have been shown to influence IgA in a number of trials. The resident Bifidobacteria and Lactobacilli in the gut can offer resistance to colonization by other potentially pathogen microbes, thereby functioning as part of the gut defense barrier. Others studies have documented the secretion of substrates with antimicrobial properties and secretion of mucins as part of the intestinal barrier that can inhibit the adherence of pathogenic bacteria.

Animals that received *L. casei*, *L.bulgaricus* and *L. acidophilus* have been shown to activate production of macrophages and enhance phagocytosis.

Treatment with competitive exclusion probiotic containing *E. coli* strain reduced fecal shedding of both O157 and O111 but not O26 zoonotic STEC in weaned calves. Treatment with different probiotic strains has had variable effects on fecal shedding of STEC in cattle. Daily treatment of finisher beef cattle with direct-fed microbials, such certain strains of *Lactobacillus acidophilus*, reduced fecal shedding of O157 STEC by over 50%.

4. Use of probiotics for restoring the indigenous microbiota reduced by use of antibiotics

Over time individuals microbiota is stable, however in some cases this stability can be altered due to external perturbations. The use of antibiotics is the major factor that can perturb the composition of the microbiota in the microbial system. One of these consequences of this effect has been the increase of antibiotic resistant pathogens. The bacterial diversity is decreased and sometimes a long-term normalization of the microbial composition of the intestinal ecosystem is needed, while some taxa among individuals do not recover even months after treatment. There is a reduced resistance to colonization because the established gut bacterial community reshapes after treatment with antibiotics, therefore the foreign microbes can cause permanent changes in the structure of the microbiota and varying states of diseases. The microbiome can become the reservoir of antibiotic resistant genes, by repeated use of antibiotics in both humans and animals production.

Some European countries have observed a reduction in the number of antibiotic-resistant pathogens following a reduction in the number of prescribed antibiotics.

Probiotics could beneficially improve the intestinal microbial balance of the host animals. The overuse or misuse of antibiotics has been linked to a certain eradication of the native microbiota. Probiotic can be used to restore the natural microbial balance and attach to the mucosal wall, adjust to immune responses and compete with pathogenic bacteria for digestive enzymes. They can stimulate synthesis of B-group and produce inhibitory compounds like volatile fatty acids and hydrogen peroxide that inhibit the growth of harmful bacteria, enhancing the host's resistance to enteric pathogens.

Several studies have demonstrated probiotic effects on a variety of gastrointestinal and extraintestinal disorders, including prevention and alleviation symptoms of diarrhea and antibiotic associated diarrhea. Some probiotic preparations have been used to prevent diarrhea caused by antibiotics, or as part of the treatment for antibiotics-related

dysbiosis. The use of probiotics preparations concomitantly with the antibiotics use for the animal could reduce the period of the diarrhea of the animals and consequently the shedding of STEC for the soil and runoff.

5. Stimulation of the immune system using probiotics

The immune system, particularly the mucosal immune system, has developed an intricate connection with microbiota. Certainly, it has been occurred because the mammals have coevolved over millions of years with the microbiota. During the development of the innate and adaptive immune systems the interactions with microbiota have been required. Some studies have shown that germ-free mice have reduced gut secretion IgA, defects in development of gut associated lymphoid tissue, and smaller Peyer's patches and mesenteric lymph nodes. When a specific commensal bacteria colonize the mucosal surfaces in response to colonization there is secretion of IgA that has been one particular plays a fundamental role in mucosal immunity.

The molecular patterns are used by innate immune system recognizes general microbe associated to them that are present across diverse lineages of bacteria such as components of the bacterial cell wall lipopolysaccharide and peptidoglycan and flagellin. Toll-like receptors (TLRs) are of the some proteins that are used by the host to recognize such antigens. The gut and mucosal immune systems do not form regularly if TLRs are not present or are mutated. For promoting immunological tolerance and suppressing inflammatory response the commensal bacteria appears to be much important and this interactions also occurs through TLRs. Other mechanism to recognition of commensal microbes by the immune system is the NOD-like receptors (NLRs) also recognize microbial molecules and can form oligomers (inflammasomes) that serve as sensors of damage associated patterns. Deficiency of NLRP6 results in reduced IL-18 levels, an altered composition of the microbiota and intestinal hyperplasia.

The commensal microbiota has programmed the adaptive immune system and microbes have been to impact the differentiation of T cell population, which can be not only determined by self or non-self-discrimination mechanisms, but also educated by commensal bacteria are also capable of modulation the host innate immune system to promote their own fitness in the intestinal niche. Some studies have shown that immunodominant determinants have been a mechanism by which commensal bacteria gain an advantage in the gut environment. On the other hand, the question that was not answered yet is whether reduced eukaryotic, parasite load is increasing autoimmune disease incidence by allowing immunological activity to be redirected in disruptive way, in absence of a coevolved engagement with microbes.

Some studies have demonstrated that inflammation suppressing indigenous bacteria are certain species strains of *Lactobacillus* and *Bifidobacterium* and those are also the main bacteria used in the production of probiotics. The use of indigenous microbiota can be a strategy to mitigate inflammation. In addition, the use of probiotic bacteria can affect the composition of the microbiota, but probiotics may also have more direct effects on the immune system and the permeability of the mucosa. The better the barrier effect of the mucosa the smaller the risk of translocation of pro-inflammatory components originating from the mucosal microbiota.

The effects of probiotic bacteria over the mucosal immune response may be divided into local and systemic. One example of the potential systemic efficacy are the immunological changes in breast milk, occurring after oral intake of *Lactobacillus bulgaricus*. Probably this occurs because there is a functional enteromammary link and the functional redistribution of active lymphocytes from the gut to the mammary gland and vice versa. Several studies have shown that the probiotics formulations could be used to balance the microbiota and consequently improve the immune response and the own probiotics improve the could be used the improve the immune response of animals thus reducing the colonization by *E. coli* and consequently its shedding

6. Characteristics of microbial health

Disease states are often correlated with imbalances in the gut microbiota. The valuable tool in the treatment of certain diseases is the restoring of a health microbial community by transplantation of a foreign gut microbiota and this procedure allows verifying the impact of the microbiota in determining phenotype. From the perspective that the hypothesis that changes in the microbiota are a driving force in diseases progression, the transplant experiments establish that the state can be recaptured by transmitting the microbiota can be used. Several diseases phenotypes induced by changes in host genetics cause dysbiosis in the gut. Remarkably, these phenotypes can be transferred to germ-free wild-type simply by inoculating them with the microbiota from the diseased donors. Furthermore, transplantation from a healthy to a diseased individual can help in recovering microbial balance in the gut. The bacterial composition in the patient has been significantly modified by transplanting the microbial community. This patient was harbored of *Clostridium difficile* associated disease. After two weeks the microbiota of the recipient had dramatically changed from configuration to community highly to that of the donor. The disappearance of the symptoms has been associated with the radical shift in the composition of the microbiota.

The appropriate conditions for a successful transfer of an exogenous microbiota are however not yet well understood. The manipulation of the bacterial communities in the gut can be helped by deep influence that antibiotics exert on the

microbiota. The use of the antibiotics prior to transplantation would facilitate the colonization process by reducing bacterial load, however previously studies have shown that antibiotic use for this purpose does not help increase the establishment of donor phylotypes, these results have suggested that the establishment of transplantation microbiota may be more difficult to achieve than previously thought.

Determining how to facilitate transplantation, what donors are more compatible with the patient, or what risks might be involved in this procedure remains an open problem in this field.

Antibiotic manipulated mice model are attractive as an inexpensive substitute for germ-free animals but more studies are requested to determine whether they can play this role effectively.

Under normal circumstances, commensal bacteria are an essential health asset with a nutritional function and a protective influence on the intestinal structure and homeostasis. The intestinal microbiota prevents infections and actively exchanges developmental and regulatory signals with the host primes and instructs mucosal immunity. Although the intestinal is complex and the role of most of the bacteria in providing benefit to the host is not clear, bacterial species of the genera *Lactobacillus* and *Bifidobacterium* have been shown to supply protection against enteric infections. By enhancing the beneficial components of the gut microbiota it is possible to treat various intestinal disorders and maintain host well-being. The probiotics strains can be a great partner that aid at this finality.

7. Contamination of animals by *Escherichia coli*

Escherichia coli is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. Most *E. coli* are harmless, however a small proportion are an important cause of disease worldwide. Both production of virulence factors and the clinical manifestation that they cause are used as base to classify the *E. coli* into categories. Certain *E. coli* produces a toxin, which was initially called verotoxin because of its distinct effect on Vero cells. After that the some toxins was called Shiga-like toxins, and more recently Shiga toxins (Stx), because of the close relation to the Stx of *Shigella dysenteriae* type I. Verotoxinogenic and Stx-producing *E. coli* (STEC) are referred as strains producing this family of toxins.

STEC are commonly found in a wide range of farm and wild animal species and, for the most part, usually do not cause disease in animals. However, strains of subset of STEC are responsible of oedema disease in pig (EDEC) and another group of STEC non-O157 enterohaemorrhagic *E. coli* (EHEC) cause dysentery in young calves. Certain STEC are zoonotic. The main way of contamination in humans is related with the ingestion of foods contaminated with the zoonotic bacteria, and clinical signs include watery diarrhea, haemorrhagic colitis (HC), and/or haemolytic uraemic syndrome (HUS). These strains were originally named enterohaemorrhagic *E. coli* because of the associated clinical signs.

The production of bacteriophage-encoded Stx is the common feature of all STEC and these toxins belong to one of two main families, each with several variants. There are over 200 serotypes that can express Stx and also within most serotypes both Stx-positive and Stx-negative strains can be found.

The serotype O157:H7 was definitely linked for the first time to several major outbreaks of HC and HUS in the United States of America (USA) and Canada. Worldwide has been observed the occurrence of zoonotic STEC and in most industrialized countries O157:H7 has been maintained as predominant serotype.

Cattle are the main reservoir of zoonotic STEC throughout the world. The advent of selective media and kits for the rapid identification of O157:H7 strains has permitted a more accurate assessment of the role of this serotype in human disease outbreaks and the transmission of the infection from animal reservoirs. However, there are no similar tests for identification of zoonotic non-O157 STEC and other STEC, which are found in the intestine tract of animals but have not yet been implicated in human infections. This situation certainly has impeded many studies of geographical distribution of these strains, the mode of transmission to humans, and the prevalence of these strains in human outbreaks and in animal reservoirs. Certainly, the prevalence of non-O157 STEC is underestimated it because many laboratories have used selective media for the specific detection of O157:H7 therewith much information about the importance of non-O157 STEC is unknown.

The cattle's feces carrying STEC can contaminate surface drinking waters, such as ponds, rivers, lakes, and ground water. Other source of contamination can be by utilization these feces as fertilizer. Contamination of drinking troughs may originate from the water source or can occurs following fecal contamination or often, when troughs are covered, following oral contamination of the drinking water by cattle carrying STEC in their tonsils. The survival time of STEC in sediments or from drinking troughs can be by several months.

Other sources of contamination can be by wild bird or mammalian feces in which run-off of contaminated water or slurries can contaminate the feeds, such as grain pellets, soybean meal, silage grasses, and grass hay. Feeds may also be contaminated during transport by truck to a feed. The contamination of feed troughs may occur through saliva or defecation in the troughs by cattle, wildlife rodents, birds, or flies.

Pen floors, water troughs, feed, pastures can become contaminated as resulted of the fecal contamination by cattle living in the environment and O157 STEC may persist for more than two years in a farm environment. The bacteria are influenced by the condition of the environment. For instance, calves kept indoors in pens continued to shed O157 STEC for four months, whereas no shedding of O157 STEC was detected over a period of six months in calves on the same

farm kept on pasture. It has happened probably due to a reduced exposure of the pasture-raised calves to the bacteria. Other situation that has showed that may affect the STEC persistence is that cattle kept in feedlot pens with wet, muddy floors present a higher prevalence rate of shedding of O157 STEC than cattle raised in pens under normal conditions.

The risk factors that have been associated with contamination and infection of animals with O157 STEC include age, weaning, movement of animals, season, feed composition and the ability of the bacteria to persist in the environment. Some studies have showed that the fecal shedding was higher in dairy calves at weaning than before weaning. Certainly, the mother's milk hinders the colonization by STEC on the gut of calves. On the other hand the feed intake different of milk also may increase the bacteria intake.

The animal reservoir should be the first target to reduce the contamination by STEC. The eradication of zoonotic STEC-positive farm animals would be practically impossible due to the high prevalence rate of O157 STEC, the transient nature of the infection and on the other hand is very difficult to detect low numbers of zoonotic STEC found in animal feces. Certainly the better effect would be to reduce intestinal colonization of STEC into animals and thus consequently would be reduced the shedding of these bacteria on the farm.

All efforts must be concentrated to reduce or minimize STEC contamination of water sources used for human consumption and recreational activities of food crops used for human consumption and of meat, meat products and milk. Undoubtedly, it would minimize the possibility of infection of humans through direct contact with animals. At the same time, measures should be taken to limit the persistence of STEC in the farm environment.

Different strategies have been attempted to reduce the intestinal colonization of cattle by zoonotic STEC, including vaccination, administration of bacteriophages and modification of the diet. The vaccination can result in decrease fecal shedding in experimentally infected cattle and in clinical trials in feedlot cattle, demonstrating the potential benefits of such approach. On the other hand, this approach usually requires some optimization as fecal shedding was not reduced after administration to the same vaccine to feedlot cattle in commercial operations. Other strategy can be the use antibody collected from eggs immunized with specific virulence factors from STEC. This approach resulted in a decrease in the duration and level fecal shedding of O157 STEC in experimentally infected sheep.

Other strategy that could be used to reduce the STEC level on the farm could be the use of antibacterial viruses, commonly known as bacteriophages that specifically target O157 and appear to be able to control the growth of these bacteria under laboratory conditions and have shown good results in sheep. The problem of this strategy is to guarantee that the viruses used to reduce the STEC can be feasible.

8. Microbial ecology and pathogens

From a microbial perspective, some studies have characterized the microbiota in the gut and its connections to the host. The study of diseases, however, has been generally approached from a "one microbe-one disease" viewpoint. Microorganisms and cells such as viruses, eukaryotes and bacteria were studied under conditions in which they were believed to cause disease. However, just as the "one gene-one enzyme" outlook proved to be an oversimplification that failed to explain complex phenotypes, and some diseases might result from dysbiosis (imbalances in the microbial community) rather than the presence of a single disease-causing microbe.

The synergistic relationship between the host animal and its gastrointestinal microbial ecosystem is critical to the health and well-being of the animal and to efficient production. The benefits of the microbial population in the gut of animals are due largely to the fermentation of dietary substrates to produce volatile fatty acids and B vitamins that are absorbed by the host animal. The stimulus caused by microbial population on the immune system can reduce colonization by pathogens and consequently illnesses.

Some techniques have been developed to reduce the pathogenic bacteria on the gut such as: introduction of a normal microbial population to the gastrointestinal tract (CE); addition of a microbial supplement called a probiotic or direct-fed-microbial (DFM), that improves gastrointestinal health and the diversity of the intestinal microbial ecology and finally providing a specific limiting nutrient that allows an expand its current niche or to occupy a new niche in the gastrointestinal tract (Prebiotics). The aims of these strategies are preventing colonization by a pathogen or displacing an established pathogenic bacterial population in the gut. The probiotic competitive enhancement strategies to become more economically feasible and widely used in disease prevention because of the fears over the dissemination of antimicrobial resistance using prophylactic antibiotic in food animals.

Studies, where probiotic have been supplemented in the ration of the animals have been characterized by inconsistency probably due to different factors such as lack of profound understanding of the microbial ecology or the gastrointestinal tract and also by conditions that would promote the growth of pathogens and the probiotic organism utilized. On the other hand, some probiotics are isolated from different sources and therefore not ecologically suited for life in the anaerobic gut. Some studies have shown different antagonistic interactions among some probiotics species, as well as quality control issues. All these aspects have produced results that are in many cases unreplicable.

9. Use of probiotics in animals

The use of probiotics in animal feeding could be enhanced by a various preliminary in vitro screening such as antimicrobial activity, survival in the gastrointestinal tract, adhesion studies and antibiotic susceptibility usually are the main probiotic properties that should be analyzed to assess functionality and safety.

Probiotic activity could be to genera, species, or strains. An approach in probiotic application could be the use of mixture of strains belonging to different genera or species.

Some features as dose, timing and duration of the administration of probiotics may be a factor affecting efficacy in acute infectious diarrhea, higher dose of probiotic given for short period of time seems to be more effective than lower doses. Therapy using *Lactobacillus* species at early treatment and long period of administration induce better and long-lasting improvement in newborn than children. Another determinant may be the age of the animals that during early life, colonization patterns are instable and newborn animals are then susceptible to environmental pathogens. As the bacteria can modulate expression of genes in epithelial cell, the initial colonization is of great importance to the host thus creating a favorable habitat for them.

All ruminants have *four*-compartment *stomach* - rumen, omasum, reticle and abomasums and when these animals born they have the abomasums extremely big in consequence by kind of food that the animals intake as milk. With the development of the animals, they become ruminants and under these circumstances occur the installation of ruminal microbiota in the gut and the distention of the organ due the fiber intake. The microbiota from rumen and bowel is acquired through the contact of cattle with the cow or other animal and by grass intake.

All feed as proteins and fibers are converted to organic acids, ammonia and amino acids by actions of microorganisms. The majority of amino acids are synthesized in the rumen itself; however, when the animals' needs are not supplied with essential amino acids, they must be provided to them through their ration or in injectable form.

The decreasing of ruminal microbiota can be caused by different factors such as dry grasslands, pastures in budding and seasonal changes. Others factors can also decrease the microbiota in rumen, as antibiotics and environment changes may occur at auctions expositions and pre-slaughter.

The cattle industry has used various types of probiotics for many years and some of these have been primarily utilized to increase growth rate, milk production, or production efficiency. In the last years the attention has seen with cattle health. The reduction of shedding of *E. coli* O157:H7 was demonstrated in sheep and another study found that feeding probiotics provided neither benefit nor detriment in regard to food-borne pathogen levels in cattle.

The prevention of diarrhea occurrence can be realized by growth of rumen bacteria into ruminants before the establishment of pathogen in these animals. The probiotics microorganisms most used belong to the groups of lactic bacteria as *Aerococcus*, *Atopobium*, *Bifidobacterium*, *Biochothrix*, *Carnobacterium*, *Enterococcus*, *Lactobacillus* and *Weissella*. The lactic bacteria are Gram-positive, anaerobic, catalase negative, fermentative bacteria that are associated with the production of lactic acid from carbohydrates. Species of *Lactobacillus*, *Lactococcus*, and *Streptococcus thermophilus* are included in this group. For ruminant usually are used ruminal bacteria as *Ruminobacter* and *Succinobivrio*. The other microorganisms commonly used are the yeast. They are unicellular microorganisms that have the capacity of survive in medium with large spectrum of pH, saline or without oxygen. The *Saccharomyces boulardii* has been extensively tested in human's trials and *Saccharomyces cerevisiae* in animals, where they showed promising results.

The *Lactobacillus* is constituted by cells that vary long and thin to short and curves with 1.5-6.0µm length and 0.6-0.9 width. The optimal growth temperature is 45°C and pH 5.5-6.0. At moment 56 species *Lactobacillus* species are known and those used as additive are, inter alia, *L. acidophilus*, *L. rhamnosus* and *L.casei*.

The genus *Bifidobacterium* includes 30 species, many of these 10 are from human's dental caries, vagina and feces, 17 are from animal origin, two are from wastewater and one of fermented milk. These bacteria have optimal growth between 37°C and 41°C and minimal growth among 25°C and 28°C at pH 6-7. The *Bifidobacterium*, *Bifidobacterium animalis*, *Bifidobacterium lactis*, *Bifidobacterium longum* species have probiotics characteristics and are also capable of fermenting complex carbons.

Some species of *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus cereus* are Gram-positive rods. The *Bacillus* are the only that form spores and thus allowing these strains to be used in adverse conditions mainly in high temperature.

A *Lactobacillus acidophilus* culture reduced *E. coli* O157:H7 shedding by more than 50% in finishing cattle and another study was demonstrated that reduction of fecal shedding of the same microorganism in cattle from 46% of animals to 13%. Others studies found that the feeding of this DFM reduced the isolation of *E. coli* O157 from hides of cattle by up to 75% in the highest dose reduced other microorganism shedding in the feces by 50%.

The genus *Enterococcus* belongs to the LAB groups. Enterococci are found naturally in food products. These microorganisms are normal human and animal commensals. *E. faecium* and *E. faecalis* are the most common in the human gastrointestinal tract while in animals. These microorganisms are used as starter cultures in food products, such as cheese, as probiotic cultures for humans and animals and as silage additives. Strains belonging to *E. faecium* have a long history of apparent safe use in industrial and agricultural applications.

Probiotics preparations for use in animals are typically comprised of individual species or mixtures of lactic acid bacteria (LAB), yeasts, and are not even necessarily originally isolated from animals. Probiotics can be categorized as

live cultures of yeast or bacteria. The most commonly used bacterial strains remain *Bifidobacteria* and *Lactobacillus* and the products where these bacteria have been applied have demonstrated production efficiency. This genus is associated with good health status of the host. There is a general belief that bifidobacteria are helpful in maintaining appropriate balance of the microbiota in the GIT, reducing the risk of pathogen infection. Several species are host specific and are very promising probiotics even if strain specific. They are frequently used in food and pharmaceutical preparations and their applications in animal feeding is increasing. Due the long history of safe use of bifidobacteria, many species are proposed for qualified presumption of safe.

In order to prevent post-weaning *E. coli* diarrhea in pigs, early weaning procedures and antibiotics are often used, yet this disease is still a significant problem for the swine industry. Some studies have found that the use of *Lactobacillus casei* culture had no impact on adherence of a pathogenic *E. coli* to the jejunum of experimentally infected conventional and gnotobiotic pigs. However, the mixture of the *L. casei* culture with maltodextrins promoted a significant reduction in gnotobiotic pigs. Other study has reported that the use of spores of *Bacillus licheniformis* together with *Bacillus toyoi* has resulted at effective reducing of diarrhea, mortality and weight loss in a field study involving 256 weaned pigs. *Lactobacillus plantarum* DFM has promoted an increase of total gut populations of lactobacillus in weaned pigs when it has been supplemented of the diet of neonatal pigs. *E. faecium* culture from birth to weaning was reported to result in a reduction in frequency of diarrhea and in improved weight gain in weaned pigs when the cause of diarrhea was not determined.

Saccharomyces is a genus of budding yeast. Yeasts are also part of the residual microbial system of the intestinal microbiota. *Saccharomyces cerevisiae* is widespread in nature and can be found in plants, fruit and soil. *S. cerevisiae* is included in foods and beverages for its key role in fermentation processes and in health foods. Strain known as *S. boulardii* was isolated from the skin of lychees grown in Indochina. This specie does not have a taxonomic status, is considered a biotype of *S. cerevisiae* and it has been used specially in ruminants and pigs feeding.

Some studies have demonstrated that reduced mucosal adhesion by pathogens promoted by supplementation of probiotics strains in the ration is presumed to lead to reduced severity of clinical diseases by probably have diminished the colonization of pathogenic bacteria.

10. Conclusions

In recent years, human infection caused by non-O157 STEC has become frequent. Another challenge will be to more definitely identify and characterize these non-O157 STEC strains, which will allow a more thorough surveillance of the prevalence of the bacteria in animal populations, assessment of the importance of these bacterial species in humans' infections and development of effective on-farm control strategies. In this context, the probiotics use can be a great strategy to reduce the shedding and consequently the presence of these pathogens on the farm reducing thus the occurrence of infections in humans. Many studies have reported that supplementing animals infected with *E. coli* O157:H7 with *S. faecium* in the diet reduces fecal *E. coli* O157:H7 shedding. However, more effective reduction in *E. coli* O157:H7 shedding can be obtained by treatment with multiple probiotic bacteria consisting of *E. faecium*, *L. acidophilus*, *L. casei*, *L. fermentum* and *L. plantarum*. Administration of probiotic bacteria as a microbial feed supplement not only reduces fecal shedding of the pathogen by ruminants or monogastrics animals but might also improve animal meat production performance as well. Reduction in *E. coli* O157:H7 shedding by ruminants decreases the chances of meat and other food products being contaminated by the pathogen thereby decreasing the potential for *E. coli* O157:H7 outbreaks and associated losses.

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