Advances in Synbiotic Therapy in the Management of Gastrointestinal Diseases (Enteric Diseases) in Farm Animals

Imtiyaz A. Bhat, Research Scholar; Muzaffar Shaheen, PhD

Division of Clinical Medicine, Faculty of Veterinary Science, SKUAST-K, Srinagar, JK 190005, India

ABSTRACT

Background: There are various emerging scientific evidences based on research trials and therapeutic practices that ‘Synbiotics’ (probiotics and prebiotics) play an important role in animal health and nutrition. There has been a significant increase in the characterization and verification of potential health benefits associated with the use of probiotics and prebiotics.

Objective: The primary clinical effects for the application of probiotics have been reported as the ability to modulate the balance and activities of the gastrointestinal (GI) microbiota, treatment of infectious diseases including viral, bacterial and antibiotic associated diarrhea, cellular immune-modulation, lowering of serum cholesterol, improvement in lactose digestion, alleviation of allergy related disorders, reducing the risk of colon cancer and imparting the colonization resistance effect on intestinal microbiota.

Results: The probiotic therapy also known as the microbial interference therapy (MIT) has in particular drawn the interest of animal clinicians, in the treatment of enteric infections of neonatal farm and food animals eliminating the entero-pathogens selectively while building up the normal intestinal flora to flourish. This cannot be achieved by the use of gut-active antibiotics, which lack the ability to discriminate between its friends and foes. The probiotic therapy in food animals has embarked upon establishing a new non-antibiotic arena in the treatment of their several infections, which ultimately not only eliminates the possibility of development of drug resistance but also render the food animals and their produce free from xenobiotic residual effects, which enter the human food chain.

Conclusion: In this review, the current knowledge on the contribution of the gut microbiota to the host well-being has been discussed. Moreover, the available information on probiotics and prebiotics and their application in animal health, production and nutrition has been reviewed.

KEY WORDS: Synbiotics; Gastrointestinal (GI); Probiotics.

ABBREVIATIONS: GI: Gastrointestinal; MIT: Microbial Interference Therapy; NK: Natural Killer; MOS: Mannose Oligosaccharide; FOS: Fructose Oligosaccharide; SARA: Subacute ruminal acidosis; LAB: Lactic Acid Bacteria; GL: Galactosyl-lactose; CEOS: Cello-oligosaccharide; MSPB: Multispecies probiotic; CSPB: Calf specific probiotic; GL: Galactosyl-Lactose.

INTRODUCTION

Probiotics and prebiotics are potentially able to modulate the balance and biological activities of the gastrointestinal (GI) microflora and are considered beneficial to the host. The conjunctival therapeutic or auxiliary use of both is called Synbiotic. Synbiotic have been used as functional foods. However, their efficacy varies and is inconsistent because of the dynamics of the GI community. Environmental factors including diet composition, feeding practices, and farm management have been shown to strongly affect the composition and functions of the microbiota in livestock animals. Probiotics have the ability to enhance intestinal health by stimulating the development of a healthy microbiota and thus stimulating the gut colonization resistance. It is important for these prebiotics and probiotics not to disturb the indigenous population,
which has adapted to the environment of the GI tract of the host. Most of the bacterial community of GI tract of mammals is occupied by two phyla, Bacteroidetes and Firmicutes.\(^2\)\(^3\) The probiotics have beneficial effects like maintenance of intestinal homeostasis, competitive elimination of pathogens, production of antimicrobial compounds like bacteriocins, promotion of gut barrier function and immune modulation through build up of macrophages, natural killer (NK) cells, antigen-specific cytotoxic T-lymphocytes and the release of various cytokines in a strain-specific and dose-dependent manner.\(^4\) Prebiotics are non-digestible food ingredients which when consumed in sufficient amounts; selectively stimulate the growth and activity of one or a limited number of microbes in the gut, including the probiotics. The mannose oligosaccharide (MOS) and fructose oligosaccharide (FOS) are the best two examples.

**GASTROINTESTINAL MICROBES IN CATTLE**

Neonatal ruminants, including calves, kids and lambs are unique because they are physically and functionally two different types of animals with respect to their GI system at birth and after birth.\(^5\) The intestine of a newly born calf is sterile and immediately after birth colonization of the GI tract begins, and complex and dynamic microbial ecosystem is established in large intestines with high densities of living bacteria as the animal matures. Changes are observed in the GI microbiota of young calves with respect to the metabolic and physiological development of the GI tract. The immature and fluctuating gut microbiota and abrupt changes in diet, may lead to an increase in the susceptibility of young animals to pathogen colonization, and subsequent diarrhea and respiratory diseases. GI microbial communities are involved in the digestion and fermentation of plant polymers, which is of particular importance in mature herbivorous animals.\(^6\) Different microorganisms interact with one another and participate in the systematic digestion of fibrous plant material which they anaerobically ferment into end products that are in turn used as energy sources by the host.\(^7\) Numerous factors such as dietary and management factors can strongly affect the structure and activities of these microbial communities, sometimes leading to impaired health and performance in livestock animals.\(^8\) Sub-acute ruminal acidosis (SARA) is a good example of digestive dysfunction that is increasingly becoming a health problem in the livestock where there is an alteration of the microbial flora.

**THERAPEUTIC APPLICATION OF PROBIOTICS IN CALVES**

Probiotics such as *Lactobacillus*, *Saccharomyces* or *Bacillus* species generally target the lower intestine and bring about stabilization of the gut microbiota, and decrease the risk of pathogen colonization in young pre-ruminants. The well-known probiotic supplements for young calves are the Lactic Acid Bacteria (LAB) that finds application in regular feeding practices. Such probiotics have beneficial effects in that they balance the gastrointestinal tract microbiota and thus, promote animal nutrition and health. The chief health problem in neonatal calves is neonatal enteritis, manifested by diarrhea, which is treated using antibiotics that are generally used to prevent calves from scouring. However, probiotics/prebiotics have been developed as alternatives to improve animal health and productivity because of the increasing safety concerns regarding the risks of antibiotic resistance and persistence of xenobiotic residues in animal products.\(^9\)\(^10\) In calves, fed fermented milk with either mixed Lactic acid bacteria or *L. acidophilus* or *Saccharomyces cerevisiae*, reduction in the incidence of diarrhea is reported.\(^11\)\(^12\) Oral treatment with Neomycin and *L. sporogenes* in two groups of undifferentiated diarrhoeic calves had the same therapeutic effects, showing that antibiotic treatment of scouring calves can be replaced with the use of probiotics.\(^13\) In a treatment trial, the therapeutic efficacy of Norfloxacain + Metronidazole was not higher than the efficacy of *L. sporogenes* + *L. acidophilus* in a group of diarrhoeic calves.\(^14\) The adherence of pathogens to the intestinal niche decreases with early colonization by *Lactic acid bacilli* in the intestinal ecosystem. Weight gain and immunocompetence in young calves has been shown to improve with a stable microbial load of *Lactobacillus species*.\(^15\) The oligosaccharides are harnessed with specific functions in calves. The MOS are believed to block colonization of pathogens in the digestive tract. Likewise, feeding MOS in combination with spray-dried bovine serum to calves, reduced the incidence and severity of enteric diseases. MOS also prevents the adhesion of Enterobacteraeae, most notably *Escherichia coli* and *Salmonella*, to the intestinal epithelium.\(^16\)\(^17\) Another sugar like Galactosyl-lactose (GL) that is a trisaccharide (galactose plus lactose) produced by the enzymatic treatment of whey with beta-galactosidase has also been suggested to have important functions in calves. Supplementation with MOS, FOS and GL may improve the growth performance of calves in both pre-weaning and post-weaning stages.\(^18\) Cello-oligosaccharide (CEOS) also used as a probiotic in calves, was utilized by specific microbes inhabiting the calf intestines. It has been observed that CEOS feeding increases the number of butyric acid-producing bacteria belonging to *C. coccoides* and *E. rectale*.\(^19\) It is a valuable source of energy, and is also involved in the growth and differentiation of intestinal cells in the large intestines, thus improving its epithelial structure\(^20\) enhancing digestion and absorption efficiencies. An *in vivo* CEOS feeding improved daily weight gain and feed efficiency in calves during the post-weaning period which was attributed to the enhancement in ruminal fermentation. CEOS also acts as a source of nutrition for various types of microbes. The various types of probiotics used in calves also include yeast culture, Multispecies probiotic (MSPB) or Calf specific probiotic (CSPB), *Lactobacillus casei* with the effects on the increase in body weight and feed efficiency.

**AUXILIARY EFFECT OF PROBIOTICS/PREBIOTICS ON THE PERFORMANCE OF HEIFERS, LACTATING COWS, AND BEEF CATTLE**

Probiotics and prebiotics (synbiotics) for adult ruminants are mainly responsible for selective and efficient fibre digestion by...
rumen microorganisms and to ameliorate gastrointestinal disease symptoms. It has been observed in a recent study that yeast supplementation in ruminants increased dry matter intake, milk yield, rumen pH, rumen volatile fatty acid concentration, and organic matter digestibility.22 The different types of probiotics have positive effects on various digestive processes in ruminants which include cellulolytic functions, synthesis of microbial proteins, and protection of animals from gastrointestinal diseases.

Different strains of yeast mostly 
*Saccharomyces cerevisiae* are the primary forms of probiotics commonly used in dairy cows. Lactate-producing bacteria such as *Enterococcus* and *Lactobacillus*, which sustain lactic acids are less commonly used than *Streptococcus bovis*, which may represent a possible means of limiting acidosis in high-concentrate-fed animals especially feedlot cattle.23 In order to avoid the accumulation of ruminal lactate in ruminants, administration of combinations containing *Megasphaera elsdenii* or *Propionibacterium* species that utilize lactate have also been administered as direct fed microbials.24,25 The most consistent effects following the addition of yeast cultures to the diet include improved productivity in both lactating and growing animals. The mode of action of yeast-products has not yet been elucidated in detail, but is generally considered to involve changes in rumen fermentation rates and patterns. Certain strains of active dry yeast are particularly effective at raising and stabilising ruminal pH by stimulating certain populations of ciliate protozoa, which rapidly engulf starch and, thus, effectively compete with amylolytic lactate producing bacteria.26,27 Yeast has the potential to alter the fermentation process in the rumen in a manner that reduces the formation of rumen gas. The cells of *S. cerevisiae* provide growth factors for rumen microbes, including organic acids and oligosaccharides, B vitamins and amino acids which stimulate microbial growth in the rumen, thereby indirectly stabilizing ruminal pH.28 Another function of yeast in the rumen is scavenging of oxygen which creates the more anaerobic environment required by rumen microorganisms.29 Thus, yeast acts not only as probiotic but also helps other rumen community members to grow, and thus acts as a prebiotic. Yeast supplementation also increased the abundance of Lactate-utilizing bacteria such as *Megasphaera* and *Selenomonas* as well as fibrolytic groups such as *Fibrobacter* and *Ruminococcus*, thus improving cellulolytic activity as a supposed mode of action of yeast. In a large scale trial, steers fed a standard steam-flanked corn-based finishing diet containing *L. acidophilus* which showed a reduction of *E. coli* O157 fecal shedding by 57%30 and by 35% in beef cattle.31 In dairy ruminants, live yeasts have been shown to improve performance by increasing dry matter intake and milk yield.32

The use of prebiotics in cattle has some disadvantages due to the ability of ruminants to degrade most of the prebiotics; however, enhancements in rumen protective technologies such as lipid encapsulation, polymer protection, etc., may allow these compounds to be used in feedlot and dairy cattle.33 Supplementation of Sorbitol, L-arabinose, trehalose and rhamnose in cattle rumen medium displaced *E. coli* O157:H7 within 72 hours,34 thus reducing the GI tract infections.

### APPLICATIONS OF PROBIOTICS/PREBIOTICS IN PIGS

Probiotics have been used in pigs to decrease the pathogen load and ameliorate gastrointestinal disease symptoms. In neonatal pigs, it has been observed that porcine-derived CE culture of known bacterial composition reduced the mortality and shedding of enterotoxigenic *E. coli* and *Salmonella enteric serovar choleraeaus*.35 The frequency of diarrhoea was reduced with improved performance as indicated by weight gain, by daily supplementation of *Enterococcus faecium* orally to piglets from birth to weaning16 and it was observed that *Enterococcus faecium* reduced the population of *Enterococcus faecalis* and other enteropathogenic bacteria responsible for the onset of post-weaning diarrhoea in piglets.37 The mode of action of probiotics in swine showed a correlation between their administration and the decline of virulence gene expression of the resident *E. coli* microbiota of the host. In neonatal weaned pigs, supplementation of the diet with a strain of *Lactobacillus plantarum* resulted in an increase in the total gut population of Lactobacilli. A symbiotic product containing *L. plantarum*, fructo-oligosaccharide and maltodextrin reduced *E. coli* 08:K88 counts in the jejunum and colon of piglets was observed due to increased acetate concentrations in the ileum and colon.38 The *L. sorbites* significantly improved daily weight gain and reduced the levels of Enterotoxigenic *E. coli* (ETEC) in the ileum when fed directly to piglets after weaning.39 Diarrhoea induced by *E. coli* K88 in post weaning piglets was cured by supplementation of probiotic containing *L. rhamnous*, possibly via modulation of intestinal microflora, regulation of production of systemic inflammatory cytokines and enhancement of intestinal antibody defences.40

Administration of *Lactobacilli* and *Bifidobacteria* in pigs immediately after birth reduced the incidence and severity of necrotizing enterocolitis and colonization density of the highly pathogenic *Clostridium perfringens*.41 *Bifidobacterium lactis* and *Lactobacillus rhamnosus* in addition individually reduced adherence of each other and that of *Salmonella, E. coli, Clostridium* species to the intestinal mucosa in swine, thus reducing the severity of clinical diseases.42 It was observed that *Bifidobacterium lactis* HN019 reduced *E. coli* infections and post-weaning diarrhoea associated with rota virus in pigs.43 The administration of *Bifidobacterium animalis* subsp. lactis showed increased growth rate and ratio of *Bifidobacteria* to *E. coli* in the gut in weaning piglets.44 It has been reported that feeding of probiotic combinations containing *Lactobacillus, Saccharomyces* and *Pediococcus*, resulted in an increased potential to modulate IgA secretion in the gut, reduced bacterial translocation to mesenteric lymph nodes, and activated the lymphocyte population following the enterotoxigenic *E. coli* infection in swine. It was reported that feeding of probiotics containing *Bacillus* species such as *Bacillus subtilis*, and *Bacillus licheniformis* in pigs caused a reduction in scours, morbidity and mortality.45 Different types of probiotics/prebiotics were added to the diet of pigs to test their influence on gastrointestinal microbiota or on health status improvement when challenged with pathogens such as Transgalacto-oligosaccharides (TOS) included at 35 g/kg in growing pigs which resulted in a significant increase in
the fecal Bifidobacteria, Lactobacilli and enhanced saccharolytic activities in the porcine colon. Similarly, Galacto-oligosaccharide (GOS) mixture supplied at 40 g/kg of diet potentially inhibited the attachment of enterotoxigenic E. coli, Salmonella to HT29 cells, showed an increase in the density of Bifidobacterium and acetate concentration, and resulted in the decrease of pH compared with control diet (Table 1).46,47

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Zoetendal EG, Collier CT, Koike S, Mackie RI, Gaskins HR. Molecular ecological analysis of the gastrointestinal microbiota: A review. J Nutr. 2004; 134: 465-472.

2. Abnous K, Brooks SP, Kwan J, et al. Diets enriched in oat bran or wheat bran temporally and differentially alter the composition of the fecal community of rats. J Nutr. 2009; 139: 2024-2031. doi: 10.3945/jn.109.109470

3. Karlsson CL, Molin G, Fåk F, et al. Effects on weight gain and gut microbiota in rats given bacterial supplements and a high-energy-dense diet from fetal life through to 6 months of age. Br J Nutr. 2011; 106: 887-895. doi: 10.1017/S0007114511001036

4. Ashraf R, Shah NP. Immune system stimulation by probiotic Microorganisms. Crit Rev Food Sci Nutr. 2004; 54(7): 938-956. doi: 10.1080/10408398.2011.619671

5. Heinrichs AJ, Lesmeister KE. Rumen development in the dairy calf. In: Garsworthy PC, ed. Calf and Heifer Rearing. Nottingham, UK: Nottingham University Press; 2005: 53-65.

6. Fatadhll Eida MT, Nagaoka J, Wasaki J, Kouno K. Isolation and characterization of cellulose-decomposing bacteria inhabiting sawdust and coffee residue composts. Microbes Environ. 2012; 27: 226-233. doi: 10.1264/jsme2.ME11299

7. Russell JB, Rychlik JL. Factors that alter rumen microbial ecology. Science. 2001; 292: 1119-1122. doi: 10.1126/science.1058830

8. Duncan SH, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol. 2004; 70: 5810-5817. doi: 10.1128/AEM.70.10.5810-5817.2004

9. Martínez-Vaz BM, Fink RC, Diez-Gonzalez F, Sadowsky MJ. Enteric pathogen-plant interactions: Molecular connections leading to colonization and growth and implications for food safety. Microbes Environ. 2014; 29(2): 123-135. doi: 10.1264/jsme2.ME13139

10. Yamamoto S, Nakano M, Kitagawa W, et al. Characterization of multi-antibiotic-resistant Escherichia coli isolated from beef cattle in Japan. Microbes Environ. 2014; 29: 136-144. doi: 10.1264/jsme2.ME13173

11. Agarwal N, Kamra DN, Chaudhary LC, Agarwal L, Sahoo A, Pathak NN. Microbial status and rumen enzyme profile of crossbred calves on different microbial feed additives. Lett Appl Microbiol. 2002; 34(5): 329-336.

12. Dehority BA. Cellulose digesting rumen bacteria. In Dehority BA, ed. Rumen Microbiology. Nottingham, UK: Nottingham University Press; 2003: 177-208.

13. Shaheen M, Muhee A, Zaman R. Therapeutic Efficacy of Probiotics in the management of Calf Enteritis. 29th ISVM Convention and National Symposium on “Recent Developments in Diagnostics and Therapeutics including Applications of Nanotechnology in Veterinary Medicine” February 17-19. Department of Veterinary Medicine Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University, Mumbai, India. 2011: 53.

14. Shaheen M, Munshi N. Studies on the Diagnostic and prognostic role of ECG in calf Diarrhoea and therapeutic trial
study with reference to Probiotics. International conference on food and Agriculture science and International conference on bioengineering and biotechnology (ICAFS-ICBB-2017) Lahore, Pakistan. 2017.

15. Shaheen M. Synbiotic therapy. In: Health Management and Disease Control Practices for Dairy Cattle. New Delhi, India: Narendra Publications; 2017.

16. Al-Saiady MY, Al-Shaikh A, Al-Mufarrej SI, Al-Showeimi TI, Mogawer HH, Dirrar A. Effect of chelated chromium supplementation on lactation performance and blood parameters of Holstein cows under heat stress. Anim Feed Sci Technol. 2004; 117(3-4): 223-233. doi: 10.1016/j.anifeedsci.2004.07.008

17. Hartemink R, Van Laere KJ, Rombouts FM. Growth of enterobacteria on fructo-oligosaccharides. J Appl Microbiol. 1997; 83: 367-374. doi: 10.1046/j.1365-2672.1997.00239.x

18. Benyacoub JF, Rochat KY, Saudan I, et al. Feeding a diet containing a fructooligosaccharide mix can enhance Salmonella vaccine efficacy in mice. J Nutr. 2008; 138: 123-129.

19. Heinrichs AJ, Jones CM, Elizondo-Salazar JA, Terrill SJ. Effects of a prebiotic supplement on health of neonatal dairy calves. Livest Sci. 2009; 125: 149-154. doi: 10.1016/j.livsci.2009.04.003

20. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 2009; 294: 1-8. doi: 10.1111/j.1574-6968.2009.01514.x

21. Prive SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett. 2002; 217: 133-139. doi: 10.1111/j.1574-6968.2002.tb11467.x

22. Desnoyers M, Giger-reverdin G, Bertin C, Duvaux-Ponter C, Sauvant D. Metaanalysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. J Dairy Sci. 2009; 92: 1620-1632. doi: 10.3168/jds.2008-1414

23. Nocek JE, Kautz WP. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and post-partum dairy cattle. J Dairy Sci. 2006; 89: 260-266. doi: 10.3168/jds.S0022-0302(06)72090-2

24. Fuller R. Probiotics in man and animals: A review. J Appl Bacteriol. 1989; 66: 365-378.

25. Kliewe AV, Hennessy D, Ouwerkerk D, Forster RJ, Mckieand RI, Attwood GT. Establishing populations of Megasphaera elsdenii YE34 and Butyrivibrio fibrisolvens YE44 in the rumen of cattle fed high grain diets. J Appl Microbiol. 2003; 95: 621-630. doi: 10.1046/j.1365-2672.2003.02024.x

26. Al-Saiady MY. Effect of probiotic bacteria on immunoglobulin concentration and other blood components of newborn calves. J Anim Vet Adv. 2010; 9: 604-609.

27. Brossard L, Chaucheyras-Durand F, Maichea-Doreau B, Martin C. Dose effect of live yeasts on rumen microbial communities and fermentations during butyric latent acidosis in sheep: New type of interaction. J Anim Sci. 2006; 82: 829-836. doi: 10.1017/ASC200693

28. McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA, Wilkinson RG. Food additives. In: Greenhalgh JFD, Morgan CA, Sinclair LA, Wilkinson RG, eds. Animal Nutrition. 7th ed. Harlow, UK: Pearson Education Ltd; 2011: 594-607.

29. Dehority BA. Numbers, factors affecting the population and distribution of rumen bacteria. In: Dehority BA, eds. Rumen Microbiology. Nottingham, UK: Nottingham University Press; 2003: 265-294.

30. Younts-Dahl SM, Osborn GD, Galvean ML, Rivera JD, Lonneragan GH, Brashears MM. Reduction of Escherichia coli O157 in finishing beef cattle by various doses of Lactobacillus acidophilus in direct-fed microbials. J Food Prot. 2005; 68: 6-10.

31. Peterson RE, Klopfenstein TJ, Erickson GE, et al. Effect of Lactobacillus acidophilus strain NP51 on Escherichia coli O157: H7 fecal shedding and finishing performance in beef feedlot cattle. J Food Prot. 2007; 70: 287-291.

32. Stella AV, Paratte R, Valnegri L, et al. Effect of administration of live Saccharomyces cerevisiae on milk production, milk composition, blood metabolites, and faecal flora in early lactating dairy goats. Small Ruminant Research. 2007; 67: 7-13. doi: 10.4315/0362-028X-70.2.287

33. Callaway TR, Edrington TS, Anderson RC, et al. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim Health Res Rev. 2008; 9: 217-225. doi: 10.1017/S1466252308001540

34. de Vaux A, Morrison M, Hutkins RW. Displacement of Escherichia coli O157:H7 from rumen medium containing prebiotic sugars. Appl Environ Microbiol. 2002; 68: 519-524. doi: 10.1128/AEM.68.2.519-524.2002

35. Genovese KJ, Anderson RC, Harvey RB, Nisbet DJ. Competitive exclusion treatment reduces the mortality and fecal shedding associated with enterotoxigenic Escherichia coli infection in nursery-raised neonatal pigs. Can J Vet Res. 2000; 64: 204-207.

36. Zeyner A, Boldt E. Effects of a probiotic Enterococcus fae-
37. Vahjen W, Taras D, Simon O. Effect of the probiotic Enterococcus faecium NCIMB10415 on cell numbers of total Enterococcus spp., E. faecium and E. faecalis in the intestine of piglets. *Curr Issues Intest Microbiol.* 2007; 8: 1-8.

38. Nemcová R, Bomba A, Gancarciková S, et al. Effects of the administration of lactobacilli maltodextrins and fructooligosaccharides upon the adhesion of *E. coli* O8:K88 to the intestinal mucosa and organic acid levels in the gut contents of piglets. *Vet Res Commun.* 2007; 31: 791-800.

39. Konstantinov SR, Smidt H, Akkermans AD, et al. Feeding of Lactobacillus sobrius reduces *Escherichia coli* F4 levels in the gut and promotes growth of infected piglets. *FEMS Microbiol Ecol.* 2008; 66: 599-607. doi: 10.1111/j.1574-6941.2008.00517.x

40. Zhang L, Xu YQ, Liu HY, et al. Evaluation of Lactobacillus rhamnosus GG using an *Escherichia coli* K88 model of piglet diarrhoea: Effects on diarrhoea incidence, faecal microflora and immune responses. *Vet Microbiol.* 2010; 141: 142-148. doi: 10.1016/j.vetmic.2009.09.003

41. Siggers RH, Siggers J, Boye M, et al. Early administration of probiotics alters bacterial colonization and limits diet-induced gut dysfunction and severity of necrotizing enterocolitis in pre-term pigs. *J Nutr.* 2008; 138: 1437-1444.

42. Collado MC, Grzeskowiak C, Salminen S. Probiotic strains and their combination inhibit in vitro adhesion of pathogens to pig intestinal mucosa. *Curr Microbiol.* 2007; 55: 260-265. doi: 10.1007/s00284-007-0144-8

43. Shu Q, Qu F, Gill HS. Probiotic treatment using *Bifidobacterium lactis* HN019 reduces weaning diarrhoea associated with rotavirus and *Escherichia coli* infection in a piglet model. *J Pediatr Gastroenterol Nutr.* 2001; 33: 171-177.

44. Modesto M, D’Aimmo MR, Stefanini I, et al. A novel strategy to select *Bifidobacterium* strains and prebiotics as natural growth promoters in newly weaned pigs. *Livestock Science.* 2009; 122: 248-258. doi: 10.1016/j.livsci.2008.08.017

45. Alexopoulos C, Georgoulakis IE, Tzivara A, Kyriakis CS, Govaris A, Kyriakis SC. Field evaluation of the effect of a probiotic-containing *Bacillus licheniformis* and *Bacillus subtilis* spores on the health status, performance and carcass quality of grower and finisher pigs. *J Vet Med A Physiol Pathol Clin Med.* 2004; 51: 306-312. doi: 10.1111/j.1439-0442.2004.00637.x

46. Tzortzis G, Goulas AK, Gee JM, Gibson GR. A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs in vivo. *J Nutr.* 2005; 135: 1726-1731.

47. Smiricky-Tjardes MR, Grieshop CM, Flickinger EA, Bauer LL, Fahey GC Jr., Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs. *J Anim Sci.* 2003; 81: 2535-2545.