

Benefits of Probiotics on Production, Health and Methane Mitigation in Ruminant Animals

¹Mebrate Getabalew and ²Ayele Negash

¹Holland Dairy Private Limited Company,
Milk Collection Point Quality Coordinator, Debrezyite, Ethiopia.

²College of Agricultural and Natural Resources Science,
Department of Animal Science, Debre Berhan University, Ethiopia

Abstract: Probiotics are viable microorganisms with beneficial health effects for animals. They are formulated into many functional animal feed. There is a growing research interest in the application and benefits of probiotics in ruminant production. The objective of this paper is to provide the potential of probiotics in animal nutrition, animal productivity and health and reduction of CH₄ emissions from ruminants. In this paper, I have reviewed current research on the benefits of probiotics on gut microbial communities in ruminants and their impact on ruminant production, health and overall wellbeing. Probiotic microbes can resist to gastric acid, bile and digestive enzymes and can attach to the intestinal wall and fight off pathogens. They have anti mutagenic effects and play a role in reducing serum cholesterol. Probiotic microbes also stimulate the immune system without causing inflammation and have anti-cancer effects. The use of probiotics in animal feeding is associated with their verified efficacy in modulation of the intestinal micro flora. Administration of probiotic strains, both individual and combined, may have a significant effect on absorption and utilization of feed, daily increase of body weight and total body weight of various animals, including sheep and goats.

Key words: Probiotic • Ruminant • Prebiotic • Methane • Health

INTRODUCTION

The joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Working Group defined probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [1]. This definition is widely accepted and adopted by the International Scientific Association for Probiotics and Prebiotics [2]. A prebiotic is a non-digestible feed ingredient that can be used to alter the composition or metabolism of the gut micro biota in a beneficial manner. In practice, prebiotics have been used almost exclusively to increase the proportion of *Bifidobacterium* and *Lactobacillus* in the gut [3]. Most of the work with prebiotics has been conducted in calves, leaving a paucity of information regarding mature cattle. For example, feeding fructooligosaccharides enhances the growth performance of veal calves by decreasing feed conversion ratios and

increasing carcass weight; however, the possible mechanisms behind these performance measures were not investigated [4].

Nowadays, the pro- and prebiotic concept is very well known regarding human applications related to preserving or restoring health. However, applications in feed are far less documented. While prebiotics should be considered a more recent concept, the history of probiotics is long and interesting. While ruminant animals play an important role in sustainable agricultural systems [5], they are also an important source of greenhousegas (GHG) emissions [6]. Regardless of the ruminant species, the largest source of GHG emissions from ruminant production is methane (CH₄), with more than 90 percent of emissions originating from enteric fermentation [7].

For many years, nutritionists have been interested in manipulating the microbial ecosystem of the rumen for improving feed utilization, therefore animal production and health, as well as, in more recent years, safety and

quality of food products from ruminants. These goals can be achieved by facilitating desirable fermentation, minimizing ruminal disorders and excluding pathogens. Antibiotics, probiotics and prebiotics have been studied with the objective to manipulate the microbial ecosystem and fermentation characteristics in the rumen and the intestinal tract of livestock animals [8]. In adult ruminants' yeasts may be used as probiotics to improve rumen fermentation. The most common prebiotics are oligosaccharides, which are non-digestible carbohydrates [9]. Nowadays, probiotics are widely used as feed additives in livestock animals and have been defined as non-pathogenic microorganisms. Objective of their use is to improve production performance and disease prevention through maintenance of a healthy gastrointestinal environment and improved intestinal function [10].

Research on probiotics and prebiotics has developed as a collaborative study domain between the fields of food and feed with medicine and pharmaceuticals. There are also a number of application studies for cattle; however, few have been discussed in association with the dynamics of the inherent microorganisms. This review explored the better usage of probiotics and prebiotics to improve ruminant performance by discussing the possible impacts of the applications of probiotics and prebiotics on the ruminant-specific GI microbial community. Therefore this review employed to assess the role of probiotics on animal health, production and methane mitigation of ruminant animals.

Effects of Probiotics on Animal Production: The significant effects of probiotics in human and domestic animal health have been well documented. Probiotics have favorable effects on FCR, WG, milk yield, gastrointestinal microbiota, pH and intestinal immunity as well as animal health status [11]. A study reported that probiotics given to sheep had increased feed intake and growth performance [12]. A small ruminant study determined that increased number of cellulolytic bacteria may improve growth rate, nutrient digestibility and fermentation process [12]. Probiotics containing *S. cerevisiae* and *E. faecium* fed to cattle had increased milk fat concentration due to increased production of volatile fatty acids (VFAs) [13].

Effects of Probiotics on Health: Several authors have revealed that microbial populations can support the animal's defense mechanism towards pathogens by

stimulating the gut immune response [11]. This may strengthen the immune systems reaction by enhancing phagocytic activity and the production of antibodies [14]. The basic purposes of immunomodulation in domestic animals include: to initiate powerful and persistent immune system responses towards infectious agents, to modulate the maturation of acquired and innate immunity during the neonatal period and in young disease sensitive animals. Also to augment local defensive immune responses at susceptible sites such as in dairy cattle (mammary gland) or in young animals (gut), to overcome the immunosuppressive effects of stress and environmental pollution [15]. Gut health refers to the health status of the upper and lower gastrointestinal tract; with possibly more emphasis on the lower GI tract. The main function of gut is to stabilize nutrients, water and electrolyte proportions, mucus secretion, cytokine expression and immune system development [16, 17].

Probiotic as Nutraceutical: The term "nutraceutical" can be defined as any food or food particles that play an essential role in maintaining normal body function that provides health benefits, including the prevention and treatment of a disease [18]. Nutraceutical are obtained from dietary supplements (probiotics, prebiotics, symbiotic, organic acids, clay minerals, exogenous enzymes, recombinant enzymes, nucleotides and polyunsaturated fatty acids), isolated nutrients (vitamin, mineral, amino acids, fatty acids) and herbal products (herbs or botanical products) [18].

Probiotics in Animal Breeding: There is plenty of evidence that indicates that improving individual animal performance reduces CH₄ produced per unit of product. It is also possible that some animals have intrinsic lower CH₄ emissions per unit of intake than others at the same level of performance. In trials with grazing sheep, Pinares-Patiño *et al.* [19] identified some animals as 'high' and 'low' emitters per unit of feed intake in a single trial and then confirmed in a second trial that these differences persisted when the same type of diet was fed. The reasons why particular animals emitted less CH₄ per unit of feed intake in these trials is not known, but it does raise the possibility of genetic differences between animals in CH₄ production. Breeding animals with higher levels of individual performance will counteract the adverse consequences for CH₄ production resulting from increases in cattle numbers in livestock producing regions.

Farm animals are exposed to the environment-related stress (e.g. rearing methods, diet, etc.). Various factors may cause disturbance of balance in the intestinal ecosystem and may become risk factors for pathogenic infections. Regardless of the species, animal health is crucial for the production chain. The use of probiotics in animal feeding is associated with their verified efficacy in modulation of the intestinal microbiota. Administration of probiotic strains, both individual and combined, may have a significant effect on absorption and utilisation of feed, daily increase of body weight and total body weight of various animals, including sheep, goats [21], cattle and horses [22].

Effect of Probiotics on Methane Mitigation: While ruminant animals play an important role in sustainable agricultural systems Eisler *et al.* [5], they are also an important source of greenhouse gas (GHG) emissions [6]. Regardless of the ruminant species, the largest source of GHG emissions from ruminant production is methane (CH_4), with more than 90 percent of emissions originating from enteric fermentation [7]. Methane emitted from ruminants is one of the major greenhouse gases that have a global warming potential 25 times higher than that of carbon dioxide [22]. Although hydrogen (H_2) is one of the major end products of fermentation by protozoa, fungi and pure monocultures of some bacteria, it does not accumulate in the rumen, because it is immediately used by other bacteria which are present in the mixed microbial ecosystem [23]. Methane is produced as a by-product of anaerobic fermentation in the reticulo-rumen of ruminants in a large part, due to the activity of methanogen archaea. Due to the complexity of the rumen microbial ecosystem, other microorganisms also regulate and alter methane production [24]. Globally, ruminants produce approximately 80 million tons of methane annually which accounts for nearly 28 per cent of anthropomorphic greenhouse gas emission [25].

There is evidence that shows that improved grass cultivars can increase animal performance without changing the quantity of feed consumed [26]. This would imply a reduction in CH_4 production per unit of product and per animal. One option that should be explored is the development through breeding of tropical grass cultivars containing high levels of water soluble carbohydrates to increase animal performance and reduce CH_4 per animal as has been shown with ryegrass genotypes in the UK [27]. Among the different strategies studied, one promising method is the manipulation of biochemical pathways

existing in the rumen to produce less methane. Use of direct-fed microbials (DFM) or probiotic is one of the possible options to manipulate it. They are an accepted alternative to the use of antibiotics and chemical substances that may induce a risk of antibiotic resistance and residues in animal products. However, to date there is little evidence to suggest the efficacy of DFM to control the production of methane in ruminants [28].

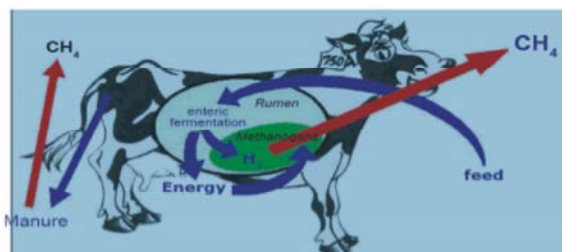


Fig. 1: The formation of methane in the rumen

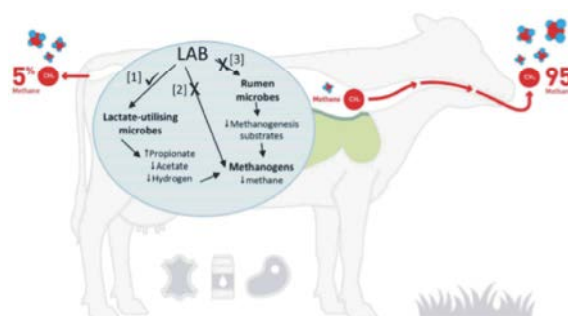


Fig. 2: Potential pathways that could be modulated by LAB to decrease CH_4 production [29].

Classification of Probiotics: There is an array of microorganisms used as probiotics, which can be classified as follows Bacterial vs non-bacterial probiotics: With the exception of certain yeast and fungal probiotics, most of the micro-organisms used are bacteria. Examples of bacterial probiotics are several species of *Lactobacillus* [30]. Non-bacterial (yeast or fungal) probiotics include *Aspergillus oryzae* and *Candida pintolopesii* [18]. Spore forming vs Non-spore forming probiotics: Although non-spore forming *Lactobacillus* and *Bifidobacterium* strains predominated initially, spore forming bacteria are now used such that *Bacillus subtilis* and *Bacillus amylo liquefaciens* [31]. Multi-species probiotics vs Single-species probiotics: The microbial composition of probiotic products ranges from a single strain to multi-strain or species compositions. Examples of multi-species probiotics are Poultry Star ME (contains *Enterococcus faecium*, *Lactobacillus reuteri*, *L. salivarius* and *Pediococcus acidilactici*) [32]; PrimaLac

(contains *Lactobacillus* spp., *E. faecium* and *Bifidobacterium thermophilum* and Microguard (contains various species of *Lactobacillus*, *Bacillus*, *Streptococcus*, *Bifidobacterium* and *Saccharomyces* [33].

Selection Criteria of Probiotics: Whereas selecting the probiotics, certain points must be taken into consideration: production, administration, application, colonial survival in the host and their physiological benefits. Probiotics should have the following properties in order to be effective: they must be able to produce antimicrobial property towards pathogens [34], they must have ability to adhere with intestinal epithelium and colonize the lumen of the gastrointestinal tract, they must have a positive effect on animals (non-pathogenic, non-reactive and non-toxic) [35], they must be able to withstand the gastric acidity, bile salts and digestive enzymes [36], they must have ability to reduce the incidence and severity of pathogen adhesion, they must have ability to stabilize normal gut microflora and be associated with health benefits [37].

Probable Modes of Action of Probiotics: Different probiotics exert their effects through various mechanisms not yet fully understood and presumed to be due to their action either in the gastro-intestinal lumen or the wall of the GIT. The mechanism of action of these feed additives appears to be different [38]. Probiotics help to prevent and control gastro-intestinal pathogens and/or improve the performance and productivity of production animals through various mechanisms. Closely related strains may differ in their mode of action there are increasing numbers of spore forming bacterial strains being used as probiotics. A small proportion of ingested spores are believed to germinate in the intestine of animal. However, It is not clear whether the germinated spores or the spores in its ingested form exert beneficial effects on the host. Major mechanisms of action proposed for probiotics are considered in the following sections [39]. Although the probiotics concept has been recognized for many years, their precise mode of action has not been fully elucidated. Principal microorganisms used as probiotics for ruminants are bacteria and yeasts. Their mode of action can be distinguished as detailed below

Yeast Probiotics: Live yeast is one of the most important probiotics used in ruminant nutrition. Over the last 30 years, our knowledge on the role of yeast in animal nutrition has been presented in the form of scientifically

proven arguments. Considerable research has been published which reviews the major milestones that have helped to define the action of live yeast in ruminants. Various modes of action have been proposed to explain effects that yeast cultures may have on rumen fermentation and ruminant production. Feeding of yeast stabilizes rumen pH, increases total volatile fatty acids (VFAs) and reduces ammonia concentration [40]. Yeasts may stimulate growth and enzymatic activity of cellulolytic bacteria, as well as improve microbial protein synthesis and fiber digestibility [41]. Yeast supplementation reduces the redox potential that creates better conditions for growth of strict anaerobic microorganisms, produces specific factors, e.g., vitamin B₁₂ or branched chain fatty acids, that way stimulating synthesis of microbial biomass in the rumen [42].

A meta-analysis showed no effect of yeasts on CH₄ production [43]. However, yeasts are capable to show great functional and metabolic diversity and some strains have been reported to decrease CH₄ production in vitro [44]. These results have yet to be confirmed in vivo. The mechanisms by which yeasts decrease methanogenesis have been proposed to be by increasing microbial synthesis [44].

Bacterial Probiotics: *Lactobacilli* and *Bifidobacterium* are the two genera most frequently used as bacterial probiotics. Various possible mechanisms of action have been considered. Some bacterial probiotic strains can competitively exclude pathogenic bacteria through colonization and adhesion to gut mucosa. This competition could be for receptors [45]. Bacterial probiotics antagonize pathogen growth through production of a variety of inhibitory substances for both Gram-positive and Gram-negative pathogenic bacteria. Potentially inhibitory agents may include organic acids, hydrogen peroxides and bacteriocins [46]. Probiotic bacteria can exert an immune modulatory effect through stimulation of the immune system and regeneration of intestinal mucosa [47].

Probiotics Microbes and Their Characteristics: These microorganisms are nonpathogenic and are not related to bacteria causing diarrhea. They can't transfer antibiotic-resistance genes and maintain genetic stability. Probiotic microbes can resist to gastric acid, bile and digestive enzymes and can attach to the intestinal wall and fight off pathogens. They have anti mutagenic effects and play a role in reducing serum cholesterol.

Probiotic microbes also stimulate the immune system without causing inflammation and have anti-cancer effects. In addition, they can increase bowel movement, maintain the health of mucus and improve the bioavailability of food components [48].

Labeling of Probiotics Use in Animal Feed: Label in the packaging of commercial probiotic products should provide information about content, positive effects of the products, date of expiry, dose rates, contraindications. However, commercial probiotics are often inadequately or incorrectly labeled and suggested that an ideal probiotic label “should state the organisms that are present to the strain level, correctly spell and identify the contents, state the number of live organisms and guarantee that the stated number would be present at the time of expiry” [49].

Another piece of essential information that should be present on the label is the dose rate to be used for different categories of animals. This was often neglected on the labels and. Few studies have examined the quality and authenticity of probiotics labeling and found that the labeling of commercial probiotics was very poor. The common errors in the labeling were failing to mention specific names of microorganisms in the product, failing to give number of viable microorganisms in the product, giving conflicting information, not mentioning expiry date and misspelling the microbial name [49].

Safety Factors Related to Probiotics: Although microorganisms used as probiotics in animal feed are relatively safe, precautions should be taken to protect animals, humans and the environment from potentially unsafe micro-organisms. Theoretically, risks associated with the use of probiotics in animal feed are as follows: Infection (gastro-intestinal or systemic) of the animal fed the probiotic, Infection (gastro-intestinal or systemic) of the consumers of animal products produced by animals fed probiotics, Transfer of antibiotic resistance from probiotics to other pathogenic micro-organisms, Release of infectious micro-organisms or noxious compounds to the environment from the animal production system [50].

Probiotics have excellent effects throughout the gastrointestinal tract. All these microbes are of natural origin; thus any deleterious effect is highly questionable. But probiotic registration plays a significant role in environmental safety and it has better safety records than antibiotics feed additives. Several studies have been conducted with no adverse effects being reported on animal health. Concisely, they are not transmitted from the gut to the body of animal. They are safe, have no food

transmission from animal origin to human and do not cause residual effects [51].

Bajagai *et al.* [51] have reported that probiotics formulator should emphasize 4 factors to avoid the recent allegation made on probiotic safety.

- Probiotic strains cannot be considered as 100% safe or with zero risk, like in case of drugs.
- The risk of probiotics application depends on immunity and health status of animal. Therefore, probiotics may be safe in one animal (healthy) but may not be safe in another (immune deficient).
- Each specific probiotic species cannot be evaluated based on other probiotics, as each product has their own safety and risk evaluation plan based on each case study.
- Lack of public awareness to hazardous effects of probiotics, so there is need to inform the consequences of probiotic risk to general public.

CONCLUSION

Direct fed microbial have the potential to reduce the current reliance on antimicrobials as a tool to promote health and optimize productivity in cattle. Therefore, GI health may be defined as the ability to maintain a balance of GI ecosystem. Desirable community shift may be attributed to the effect of probiotics and prebiotics, rather than autonomic change. Probiotics and prebiotics both have great potential in livestock productivity as well as human health. Mitigation of CH₄ emissions can be effectively achieved by strategies that improve the efficiency of animal production, reduce feed fermented per unit of product, or change the fermentation pattern in the rumen. Many current and potential mitigation strategies have been evaluated, but not all of them can be applied at the farm level and in many cases, the potential negative effects and associated costs have not been fully researched. Strategies that are cost effective, improve productivity and have no potential negative effects on livestock production hold a greater chance of being adopted by producers.

REFERENCES

1. FAO, 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba: Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report.

2. Hill, C., F. Guarner, G. Reid, G.R. Gibson, D.J. Merenstein, B. Pot, L. Morelli, R.B. Canani, H.J. Flint, S. Salminen, P.C. Calder and M.E. Sanders, 2014. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol. Hepatol.*, 11(8): 506-514. <https://doi.org/10.1038/nrgastro.2014.66>.
3. Gibson, G.R., H.M. Probert, J.V. Loo, R.A. Rastall and M.B. Roberfroid, 2004. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutr. Res. Rev.*, 17: 259-275.
4. Grand, E., F. Respondek, C. Martineau, J. Detilleux and G. Bertrand, 2005. Effects of short-chain fructooligosaccharides on growth performance of preruminant veal calves. *J. Dairy Sci.*, 96: 1094-1101.
5. Erezende, A.S.C., P. Trigo, A.M.Q. Lana, J.M. Santiago, V.P. Silva and F.C. Montijano, 2012. East as a feed additive for training horses. *Cienc Agrotecnol.*, 36: 354-62.
6. Jouany, J.P., 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. *Anim. Reprod. Sci.*, 96: 250-264.
7. Khalid, M.F., M.A. Shahzad, M. Sarwar, A.U. Rehman, M. Sharif and N. Mukhtar, 2011. Probiotics and lamb performance: A review. *Afr J. Agr. Res.*, 6: 5198-5203.
8. Lovett, D.K., D. McGilloway and A. Bortolozzo, 2006. In vitro fermentation patterns and methane production as influenced by cultivar and season of harvest of *Lolium perenne* L. *Grass and Forage Science*, 61: 9-21.
9. Bimrew, A., 2014. Biotechnological Advances for Animal Nutrition and Feed Improvement. *World Journal of Agricultural Research*, 2(3): 115-118.
10. Mountzouris, K.C., C. Balaskas, I. Xanthakos, A. Tzivinikou and K. Fegeros, 2009. Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with *SalmRoselli* M, Pieper R, Rogel-Gaillard C, de Vries H, Bailey M, Smidt H, Lauridsen C (2017) Immunomodulating Effects of Probiotics for Microbiota Modulation, Gut Health and Disease Resistance in Pigs. *Anim. Feed Sci. Technol.*, 233: 104-119. *onellaenteritidis*. *Br Poult. Sci.*, 50: 467-478.
11. Parvez, S., K.A. Malik, S. Ah, Kang and H.Y. Kim, 2006. Probiotics and their fermented food products are beneficial for health. *J Appl. Microbiol.*, 100: 1171-1185.
12. Seo, J.K., S. Kim, M.H. Kim, S.D. Upadhaya, D.K. Kam and J.K. Ha, 2010. Directfed Microbials for Ruminant Animals. *Asia.n-Aust. J. Anim. Sci.*, 23(12): 1657-1667.
13. Oetzel, G.R., K.M. Emery, W.P. Kautz and J.E. Nocek, 2007. Direct-fed microbial supplementation and health and performance of pre- and postpartum dairy cattle: A field trial. *J. Dairy Sci.*, 90: 2058-2068.
14. Tripathi, M.K. and S.A. Karim, 2010. Effect of individual and mixed live yeast culture feeding on growth performance, nutrient utilization and microbial crude protein synthesis in lambs. *Anim Feed Sci. Techn.*, 155: 163-171. Italy.
15. Nigatu, A., 1998. Systematics of *Lactobacillus* and *Pediococcus* isolates from fermented tef (*Eragrostistef*) and kocho (*Enseteventricosum*) and microbiological status of the baked products. PhD dissertation, Department of Biology, Addis Ababa University, Ethiopia, pp: 1-102.
16. Panesar, P.S., 2011. Fermented dairy products: Starter cultures and potential nutritional benefits. *Food Nutr. Sci.*, 2: 47-51.
17. Wang, A., H. Yu, X. Gao, X. Li and S. Qiao, 2017. Influence of *Lactobacillus fermentum* I5007 on the intestinal and systemic immune responses of healthy and *E. coli* challenged piglets. *Antonie Van Leeuwenhoek*, 96(1): 89-98.
18. Das, L., E. Bhaumik and U. Raychaudhuri, 2012. Role of Nutraceuticals in Human Health. *J Food Sci Technol.*, 49: 173-183.
19. Pinares-Patiño, C.S., M.J. Ulyatt and K.R. Lassey, 2007. Persistence of differences between sheep in methane emission under generous grazing conditions. *The Journal of Agricultural Science*, 140: 227-233.
20. Chiofalo, V., L. Liotta and B. Chiofalo, 2004. Effects of the administration of *Lactobacilli* on body growth and on the metabolic profile in growing Maltese goat kids. *Reprod Nutr. Dev.*, 44: 449-57.
21. Tefera, T.L., 2010. Commercializing dairy and forage systems in Ethiopia: An Innovation Systems Perspective. ILRI – IPMS. Working Paper No. 17.

22. IPCC, 2007. Intergovernmental Panel on Climate Change: Impacts, Adaptation and Vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change, Cambridge University Press, Cambridge, UK.
23. Moss, A.R., J.P. Jouany and J. Newbold, 2000. Methane production by ruminants: its contribution to global warming. *Annales De Zootechnie*, 49(3): 231-25
24. Morgavi, D.P., E. Forano, C.J. Martin and Newbold, 2010. Microbial ecosystem and methanogenesis in ruminants. *Animal*, 4(7): 1024-1036.
25. Beauchemin, K.A., M.O. Kreuzer, F. Mara and T.A. McAllister, 2008. Nutritional management for enteric methane abatement: a review. *Aust. J. Exp. Agric.*, 48: 21-27.
26. Woodfield, D.R. and H.S. Easton, 2004. Advances in pasture plant breeding for animal productivity and health. *New Zealand Veterinary Journal*, 52: 300-310.
27. Tegegne, A., 2018. Why Ethiopia's Dairy Industry can't meet Growing Demand for Milk. Retrieved June 05, 2019, from The Conversation: <http://theconversation.com/why-ethiopia-s-dairy-industry-cant-meet-growing-demand-for-milk-100067>.
28. Jeyanathan, J., C. Martin and D.P. Morgavi, 2014. The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. *Animal*, 8(2): 250-261. <https://doi.org/10.1017/s1751731113002085>.
29. Tsehai, G., K. Amiha and A. Berhanu, 2013. Microbial profile of Metata (fermented cheese) and the role of spices as an antimicrobial agent against spoiling microorganisms in traditional fermentation process. MSc Thesis, Haramay University, Ethiopia, pp: 1-75.
30. Yilma, Z., G.B. Emmanuelle and S. Ameha, 2011. A Review of the Ethiopian Dairy Sector. Ed. Rudolf Fombad, Food and Agriculture Organization of the United Nations, Sub Regional Office for Eastern Africa (FAO/SFE), Addis Ababa, Ethiopia, pp: 81.
31. Eyassu, S., 2013. Chemical composition and microbiological quality of Metata Ayib: a traditional Ethiopian fermented cottage cheese. *Int. Food Res. J.*, 20(1): 93-97.
32. Farnworth, E.R. and C.P. Champagne, 2015. Production of probiotic cultures and their incorporation into foods. In: *Probiotics, Prebiotics and Synbiotics: Bioactive Foods in Health Promotion*. Vol. 20 eds Watson R. R., Preedy V. R., editors. (Amsterdam: Elsevier), pp: 303-318.
33. Gilliland, E., 1987. Characteristics of Cultures used for the Manufacture of Fermented Milk Products. In "Milk- the Vital Force". Proceedings of the XXII International Dairy Congress, The Hague, September 29 -October 3, 1986. Edited by the Organizing Committee of the XXII International Congress. D. Reidel Publishing.
34. Gonfa, A., A. Howard, B. Foster, H. Wilhelm and C. Holzapfel, 2001. Field survey and literature review on traditional fermented milk products of Ethiopia. *Int. J. Food Microbiol.*, 66(3): 173-186.
35. Haile, G., 2009. The impact of global economic and financial crisis on the Ethiopian dairy industry. Impact of the global economic crisis on least developed countries' (LDCs) productive capacities and trade prospects: Threats and opportunities, Least Developed Countries Ministerial Conference, UNIDO, UN-OHRLS, 3-4 December 2009, Vienna International Center, Austria.
36. Jans, C., 2008. Identification and diversity of lactic acid bacteria responsible for spontaneous acidification of camel milk for the purpose of developing a defined starter culture. ILW Food Biotechnology, ETH Zurich.
37. Danfeng, S., I. Salam and H. Saeed, 2012. Recent Application of Probiotics in Food and Agricultural Science. DOI: 10.5772/50121.
38. Fajardo, P., L. Pastrana, J. Mendez, I. Rodriguez, C. Fucinos and N. Guerra, 2012. Effects of feeding of two potentially probiotic preparations from lactic acid bacteria on the performance and faecal microflora of broiler chickens. *Scientific World Journal*, Art. No. 562635.
39. Lodemann, U., 2010. Effects of Probiotics on Intestinal Transport and Epithelial Barrier Function Bioactive Foods in Promoting Health Probiotics and Prebiotics. Academic Press, Waltham, USA.
40. Bakr, H.A., M.S. Hassan, N.D. Giadinis, N. Panousis, D. Ostojić Andrić, M.M. Abd El-Tawab and J. Bojkovski, 2015. Effect of *Saccharomyces cerevisiae* supplementation on health and performance of dairy cows during transition and early lactation period. *Biotechnol. Anim. Husb.*, 31: 349-364.
41. Bomba, A., R. Nemcova, S. Gancarikova, R. Herich, P. Guba and D. Mudronova, 2002. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructooligosaccharides and polyunsaturated fatty acids. *Br. J. Nutr.*, 88: 95-99.

42. Chaucheyras-Durand, F., N. Walker and A. Bach, 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. *Animal Feed Science and Technology*, 145(1): 5-26.
43. Sauvant, D., 2005. Rumen acidosis: modeling ruminant response to yeast culture. In *Nutritional biotechnology in the feed and food industries* (ed. TP Lyons and KA Jacques), pp: 221-228. Nottingham University Press, Nottingham, UK.
44. Newbold, C.J., S. Lopez, N. Nelson, J.O. Ouda, R.J. Wallace and A.R. Moss, 2005. Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation *in vitro*. *British Journal of Nutrition*, 94: 27.
45. Guillot, J.F., 2003. Probiotic feed additives. *J. Vet. Pharmacol. Ther.*, 26: 52-55.
46. Schierack, P., M. Filter, L. Scharek, C. Toelke, D. Taras, K. Tedin, K. Haverson, A. Lubke-Becker and L.H. Wieler, 2009. Effects of *Bacillus cereus* var. *toyoi* on immune parameters of pregnant sows. *Vet. Immunol. Immunopathol.*, 127: 26-37.
47. Vondruskova, H., R. Slamova, M. Treckova, Z. Zraly and I. Pavlik, 2010. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: a review. *Vet. Med., (Praha)* 55: 199-224.
48. Aziz, S., W. Yan, S. Jing, Z. Hang, L. Nianzhen, X. Hengyong, Z. Qing and L. Yiping, 2018. Effect of probiotics on the meat flavour and gut microbiota of chicken. *Scientific Reports* volume 7, Article number, 6400.
49. Weese, J. and H. Martin, 2011. Assessment of commercial probiotic bacterial contents and label accuracy. *Canadian Veterinary Journal*, 52(1): 43-46.
50. Doron, S. and D. Snyderman, 2015. Risk and Safety of Probiotics *Clinical Infectious Diseases*, 60(suppl. 2): S129-S134.
51. Bajagai, Y.S., A.V. Klieve, P.J. Dart and W.L. Bryden, 2016. *Probiotics in Animal Nutrition Production, Impact and Regulation*. FAO Animal Production and Health 24: 1-89, Rome.