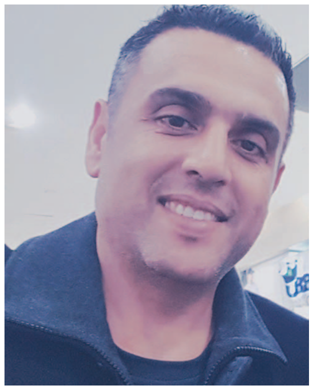
On the use of probiotics to improve dairy cattle health and productivity



Divya Krishnan[A], *Hulayyil Al-harbi*[A], *Justine Gibson*[A], *Timothy Olchowy*[A,B] *and John Alawneh*[A,C]

[A]Good Clinical Practice Research Group, School of Veterinary Science, University of Queensland, Gatton, Qld 4343, Australia

[B]Faculty of Veterinary Medicine, Department of Production Animal Health, University of Calgary, Calgary, Alberta T2N4Z6, Canada

[C]Tel.: +61 7 5460 1992, Email: j.alawneh@uq.edu.au

Abstract. Probiotics are genetically identifiable, live microorganisms that when administered in adequate amounts, confer appropriately sized health benefit (e.g. correcting dysbiosis, immunomodulatory effect) on a target host. In cattle, probiotics have shown promising results and long-term benefits in productivity when used on animals under stress. The health and production benefits of probiotics were attributed to improvement in fermentation in rumen and intestine, the stabilisation of rumen pH, and improvements in the intestinal barriers. In the bovine udder, a dysbiosis of the commensal intramammary microbiota and the presence of mastitis causing-bacteria has been linked to increased intramammary infections. Probiotic bacteria capable of biofilm formation inside the udder either serve as a barrier against pathogens or disrupt and replace biofilms formed by pathogens. Over the past two decades, several types of probiotics have been used as feed additives; however, the effect of probiotic use on disease prevention and cattle health and performance indicators, and characterisation of the immunomodulatory association between probiotic microbiota and host target system microbiota are yet to be quantified or documented.

The advent of the ban on the use of the antibiotics in agriculture in 1986 in Sweden, followed in 1999 by The Netherlands[1]. In 2003, the United Nations tripartite (World Health Organization, Food and Agriculture Organisation and World Organisation for Animal Health) released a joint report titled 'Non-human antimicrobial usage and antimicrobial resistance: scientific assessment report', which recommended strict surveillance and monitoring, and moderation of antimicrobial usage in the food-producing animal industry, specifically due to the public health implications entailed by zoonotic transmission of bacteria such as *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., and *Enterococcus* spp. and antimicrobial usage or resistance risks[2].

The report raises many issues related to antimicrobial use patterns and their implications on animal and human health. The most important question arising from the animal health perspective was how health and productivity standards, on both animal and herd levels, can be maintained while reducing (or eliminating) the needs for antimicrobials? Noting that the definition of animal- and herd-level health indicator metrics such as mortality/morbidity rates, disease incidence, immune response and feed conversion efficiency vary between animal production systems[3]. Therefore, quantifying the effect of antimicrobial usage reduction on animal health and productivity across production systems remains a challenging task[4–6].

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'[7,8], the microorganisms must be must be alive in an adequate number when administered, strains must be identified genetically and appropriately tested on target conditions and hosts[8]. The probiotic interaction with host's system microbiota (e.g. udder, rumen, intestine) results in correcting system dysbiosis[9] and controlling several infectious inflammatory conditions through antagonism and immunomodulation[10]. Lactic acid bacteria (LAB) that are well known antibacterial producers and generally recognised as safe in the food industry offer a possible alternative to conventional antimicrobials[11].

The mammary gland contains unique microbiota[12,13]. The presence of bacteria not associated with mastitis in the healthy udder reinforces the concept of commensal mammary microbiota, and the ecological structure of the healthy udder microbiota may provide an understanding of the pathogenesis of intramammary infections (IMI) and offer opportunities for developing therapeutic or prophylactic products as an alternative to antimicrobials[14]. A dysbiosis of the commensal intramammary microbiota and the presence of mastitis causing-bacteria has been linked to IMI in dairy cattle[12]. The use of probiotics is proposed to correct the dysbiosis[9]. Studies have been conducted using viable cultures of LAB as intramammary infusions to successfully treat mastitis pathogens with the same efficiency as conventional antimicrobials[15]. Direct infusion with *Lactococcus lactis* in the udder has been shown to induce a rapid and considerable innate immune response with the greatest increase in immune gene expression coinciding with peaks in somatic cell counts (SCC)[10].

In 2015, a study was conducted to determine the effect of an intramammary infusion with a LAB-based probiotic mix in healthy lactating dairy cows[16]. The mix successfully elicited a massive inflammatory/immune response in the infused quarters[16]. The magnitude of the response is particularly noteworthy as the LAB-based mix did not colonise within the udder and bacterial counts recovered from milk decreased to zero 48 h post infusion. All animals experienced an increase in SCC and swollen udder quarters. The immune response was short-lived and SCC returned to pre-infusion levels within five days. It was hypothesised that the immune profile elicited by the LAB-mix was different from a pathogen assault and may prove to be a successful non-antibiotic treatment for mastitis because of the LAB-mix's ability to produce a bacteriocin with broad-spectrum antibacterial activity against gram-positive pathogens and elicit a rapid and substantial innate immune response. The 2015 study findings compare very favourably with other therapies recently investigated to treat mastitis[10].

Probiotic bacteria capable of biofilm formation have also shown promising results in the prevention of mastitis. The biofilm formation inside the udder either serves as a barrier against pathogens[17] or disrupts and replaces biofilms formed by pathogens[18]. The latter could have been driven by interspecies interactions: high growth rates and dominance of probiotic organisms over other biofilm formers[19] and substrate competition[20,21].

A controlled, crossover study was conducted in 2018 to evaluate the safety and efficacy of LAB-based probiotic applied as a teat spray in improving SCC of lactating dairy cattle[22]. On average, milk SCC in the control group was 66% higher (1.66, 95% confidence interval (CI) 1.08–2.56, $P = 0.02$) compared with the probiotics group (Figure 1)[22]. The study concluded that the probiotic bacteria may have produced a biofilm which could have hindered the colonisation of other bacterial isolates resulting in reduced bacterial counts on the teats. Our results compare favourably with the literature[17]. More work is needed to better understand the exact mode of action of the probiotic product tested. The successful identification of inflammatory modulators (pro/pre), antibacterial peptides and development of a new biological mastitis therapy could significantly reduce the substantial economic losses incurred by the dairy industry worldwide and improve animal health, productivity and welfare while increasing food safety[23].

In cattle, probiotics used as feed additives have shown health and productivity benefits when used when animals are assumed to be under stress[24]. In lactating dairy cattle, after controlling for the effect of days in milk, and cow parity, cows ingesting probiotics have been reported to produce an average of 1.21 L/day more milk (95% CI 0.34–2.08 L/cow per day; Figure *2a*), more milk protein (0.03 kg/day; 95% CI 0.01–0.05 kg/day; Figure *2b*), numerically lower average SCC and fewer clinical cases of

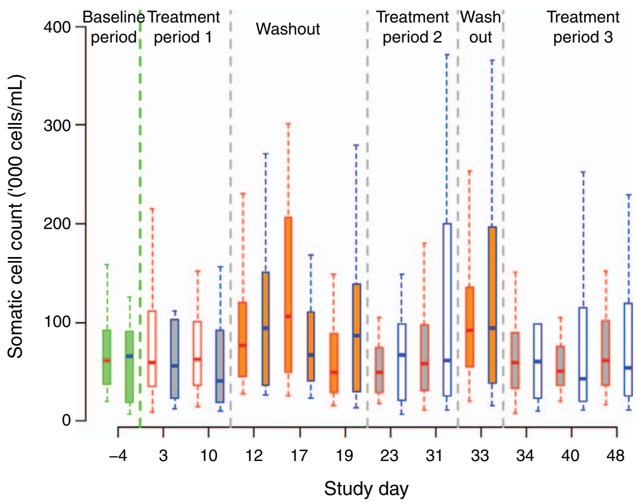


Figure 1. Box-of-whiskers plot of somatic cell count ('000 cells/mL) observed during baseline (green shaded boxes; experimental group A1 red border and red horizontal line; experimental group A2 blue border and blue horizontal line) and treatment periods (control article group in white shaded boxes; probiotics article group in grey shaded boxes; washout period in orange shaded boxes) of the study. The study design was a 3 × 2 randomised, controlled, crossover study conducted between June and December 2018[22].

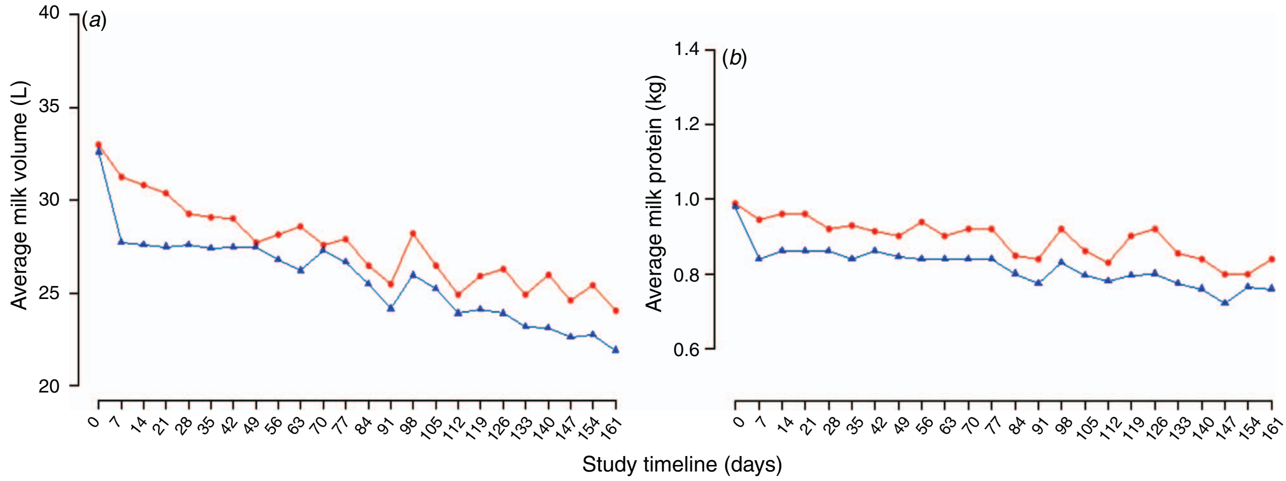


Figure 2. Line plot of average weekly milk production (L; *a*) and milk protein (kg; *b*) for the control (blue solid triangles and blue solid line) and probiotics group (red solid circles and red solid line). The results are from a randomised controlled study[25] conducted in 2018.

lameness and mastitis than the control cows[25]. Similar effects on calf health and productivity were also reported[26]. Calves on probiotics were heavier at weaning, and on average, rumen and intestinal organs' folding and crypts were more developed and more adapted compared with control calves[26]. These benefits were hypothesised to be linked to improvement in the ruminal and intestinal fermentation[27]. The current known mechanisms of action of probiotics in ruminants appears to be through a shift in the microbiota of rumen and rear-gut (small and large intestine), an improvement in fermentation or volatile fatty acids, the stabilisation of rumen pH, and improvements in the intestinal mucosal barriers through the probiotics competitively excluding pathogens and improving the local and systemic immune response[28,29].

Probiotic bacteria have also been isolated from soil[23], fermented green tea[30], the gastro-intestinal tract of various animals including poultry[31] and cattle[32]. The most common genera of bacteria used include *Lactobacillus* spp., *Bacillus* spp., *Bifidobacterium* spp., *Streptococcus* spp. and *Enterococcus* spp. The intended application of probiotic bacteria varies. Studies that use bacteria like *Dietzia* spp., and *Megasphaera* spp., are more focused on their prophylactic uses. The choice of the interventions was based on the presumed mechanism of action of the probiotic strains, e.g. bacteriocin production, lactic acid production, oxygen scavenging, immune-modulation and more generally, their ability to establish a healthier microbial composition in the gastrointestinal tract. The general intent of probiotics is to replace the need for antimicrobials, but the combination of the two have been explored by few: Click (2011) used tetracycline with *Dietzia* spp. to prevent the development of Johne's disease symptoms in calf neonates[33], and Timmerman *et al*. (2005) used a prophylactic antibiotic and probiotic mixture to improve the health and growth of veal calves[34]. In more recent years, probiotic development has shifted from bacteria to using other organisms like yeast (*Saccharomyces* spp., *Candida* spp.) and mould (*Aspergillus* spp.). It has been identified that a mixture of organisms is more effective and are generally better for prophylactic therapy[34]. Some commercial probiotics are combined with other naturally isolated compounds such as allicin (e.g. Enteroguard®- Romvac Company) and medicinal plant mixes[35], which enhance the beneficial effects of the probiotic bacteria by acting synergistically. The most commonly observed or hypothesised mechanisms include the production of inhibitory substances like bacteriocins, organic acids and hydrogen peroxide, production of biofilms by changing bacterial population of gastrointestinal tract; 'stimulating faecal shedding of coliforms, decreasing concentration of stress hormones like cortisol, increasing in number of $CD3^+$, $CD4^+$, $CD45R^+$, $CD8^+$, T cells, $WC1^+$, $CD282^+$, detoxification of blood from heavy metals like zinc, cadmium and lead'[36,37]. There is a consensus in the literature further investigations into the exact mechanism of action of probiotics is required in order to maximise the outcome benefits that may be derived from probiotics bacteria.

Conclusions

Probiotics have been proposed as a viable alternative to antimicrobials to enhance animal health and productivity. Over the past two decades, several types of probiotics have been used as feed additives, however, the effect of probiotics use on disease prevention on cattle health and performance indicators (e.g. rumen health and development, growth rate, feed conversion), and characterisation of the immunomodulatory association between probiotic microbiota and host target system microbiota are yet to be quantified or documented.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

Ms Divya Krishnan, BSc, is currently a student of veterinary medicine at the University of Queensland. She graduated in 2017 with her Bachelor of Science degree, having completed it with three majors in Biotechnology, Chemistry and Zoology. Her

research interests include epidemiology, wildlife medicine and rehabilitative medicine.

Hulayyil Alharbi, BSc, is a PhD candidate in the group of Dr John Alawneh, in the School of Veterinary Science at the University of Queensland Gatton campus. He completed his MSc in Veterinary Public Health at the University of Glasgow and is now pursuing a PhD project in bovine udder microbiome research focusing on intramammary infection and its influence on udder microbiota.

Dr Justine Gibson, BVSc PhD GCEd, has held the position of Lecturer in Veterinary Bacteriology and Mycology at the School of Veterinary Science, University of Queensland since 2010. Justine graduated with a Bachelor of Veterinary Science in 1996 and after working in veterinary practice for a number of years undertook a PhD investigating the epidemiology and basis of fluoroquinolone resistance in multidrug-resistant *Escherichia coli* and Enterobacter spp. isolated from companion animals. Her research spans the fields of veterinary microbiology and molecular biology, and includes: antimicrobial resistance and stewardship; diagnostics, the development and use of novel therapies and probiotics; infectious diseases and microbiota of wildlife; and production and companion animals.

Dr Timothy Olchowy, DVM PhD, is a graduate of the Ontario Veterinary College (Guelph) and has extensive veterinary experience in both academic and private practice as well as in the animal health industry. He is ACVIM board certified in large animal medicine with a focused interest in cattle health and medicine. His primary areas of research interest are respiratory and gastrointestinal diseases of cattle and calves.

Dr John Alawneh, BVSc PgDip Public Health MVSc PhD GCEd, is a Lecturer in veterinary epidemiology, School of Veterinary Science, University of Queensland. He has 20 years of experience in production animals' reproduction and medicine (10 year in TMR tie stall systems and 10 years in pastoral systems) and nine years' experience in research and teaching. Dr Alawneh holds a BVSc (JUST, Jordan, 1999), PgDip VPH (Massey University, NZ, 2006), MVSc Hons (Massey University, NZ, 2005), PhD – epidemiology (Massey University, NZ, 2011). In 2016 he established and led the Good Clinical Practice Research Group. His main research interests are in veterinary and molecular epidemiology, which includes: use of novel alternatives to antimicrobial therapy in veterinary medicine; use of probiotics to improve animal health and productivity; enhancing herd health, productivity and welfare through automation; and utilising smart tools as decision support systems in production animals.

Pangolins' purpose is pursuing ants not propagating peril

Pangolins, also known as scaly anteaters, appear to be a plausible link between horseshoe bats and humans in the coronavirus line of transmission. Pangolins are docile and reclusive creatures that live in tropical forests and are a native species of Southeast Asia. So how did they gain a role in disease creation and transmission?

Pangolins are the world's most trafficked mammals and are valued for for their meat and scales. Pangolin scales are made of keratin, like rhinoceros horns, and although they have no proven medicinal value they are used in traditional Chinese medicine to help conditions ranging from lactation difficulties to arthritis. Pangolins are very strong diggers and this ability to break through barriers and blockages is believed to reside in their scales.

In addition people pay up to $1000 for a live Pangolin to keep as a pet. They are very gentle and have no teeth. They carry their young on their back, like koalas, but could never be regarded as cute or cuddly. In Vietnam, pangolin flesh is an exotic food fetching up to $300 per kilo.

Many exotic animals are both poached and farmed, and subsequently eaten for novelty, therapy or for good fortune rather than for sustenance, as was the case originally. This practice, happily, is said to be slowly falling out of favour in mainland China.

Let us hope that the disastrous consequences of close contact with, and consumption of pangolins and other native species, are now fully recognised - for all our sakes - and that they are left to forage in protected tracts of tropical forest, rather than be captured and marketed in mixed markets where their viral passengers can find human hosts and subsequently cause widespread suffering and loss.

Maybe fortune cookie inserts could be printed to promote the message that good fortune follows preservation not pillage? Pangolins particularly.