Alternatives to antibiotics: probiotics, the gut microbiome and immunity

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Dr TB Barragry PhD,MSc,MVB, MRCVS, Dip ECVPT discusses the role probiotics can play in providing an alternative to antibiotics and assisting in disease avoidance

Antimicrobial Resistance (AMR) is now recognised as a major global threat to animal and human health. In the EU, AMR is estimated to be responsible for 33,000 deaths per year, and costs €1.5bn per annum, in terms of healthcare costs and productivity losses. The EU has projected that unless effective new measures and actions are put in place, AMR to second line antibiotics will be 72 per cent higher in 2030 compared to 2005. In the same period, AMR to last line treatments will more than double.

The European "One Health Action Plan" acknowledges the serious social and economic burden of AMR and has proposed that a "One Health" approach is taken, recognising the clear interconnection and interdependence between human health, animal health and the environment. The concept of "Reduce, Replace and Rethink" has been proposed by the Joint EFSA and EMA expert group as an underlying concept to be embedded in all future strategies, policies and legislation regarding antibiotic usage. The new Veterinary Medicines Regulation (EU) 2019/6 that will apply from 28 January 2022, considers AMR to human and veterinary products to be a growing health problem requiring urgent action.

The main aims of this new EU legislation is to increase availability of veterinary medicinal products, to tackle AMR, to reduce the administrative burden in registering and developing new products, and to strengthen innovation. The new legislation, *inter alia*, prohibits oral mass medication and the metaphylactic and prophylactic use of antibiotics. In this context, stimulating the development of new, alternative agents in order to reduce the reliance on antibiotics is one of the pillars of the fight against the AMR threat and is a high priority for the EMA and the European medicines regulatory network.

ALTERNATIVES TO ANTIBIOTICS

The EMA and EFSA published in 2016 a Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union and thus to lessen the resulting impacts on food safety (RONAFA). This opinion, which referred to these other alternative agents as ATAMs (alternatives to antimicrobials) was subsequently endorsed by the CVMP, and included a listing of alternatives such as vaccination, probiotics, phytochemicals etc and of other potential alternative measures (see Table 1).

Table 1.

Examples of alternatives to antimicrobials
Vaccines
Antibodies
Immunomodulations
Bacteriophages (wild-type, engineered)
Lysins
Antimicrobial peptides (e.g. bacteriocins, host-defense peptides)
CRISPR-Cas9-based products
Probiotic and live organisms (e.g. probiotics, predatory bacteria, competitive exclusion)
Prebiotics
Symbiotics
Postbiotics
Interferons
Phytochemicals
Herbals/Botanicals
Organic acids
Biocides
Teat sealants

Encouragement for the development, usage and uptake of these alternative agents is the keystone of current EU policy. A 'prevention is better than cure' approach and using these alternative compounds prophylactically as pre-emptive strikes, is now seen as a viable method of reducing outbreaks of clinical disease and thus minimising the need for antibiotic intervention. Antibiotics will always be needed and will always be necessary to treat serious outbreaks of clinical infectious diseases. What is at stake here is reducing the frequency of their usage, and indeed if possible, to prophylactically head off outbreaks of disease. The usefulness of any antibiotic is inversely proportional to the frequency of its usage.

In this regard the use of broad spectrum oral antibiotics is a particular problem insofar as it is a very blunt instrument which affects not only gut pathogens, but also the resident population of beneficial commensals. Oral broad spectrum antimicrobials in effect 'asset strip' the gut microbiome of more organisms than is necessary, including the vital beneficial ones, from which the microbiome may take time to recover. Thus, indiscriminate oral antimicrobial usage can be regarded as a 'subtractive approach' in terms of the gut microbiome, whereas seeding the gut with beneficial organisms, such as by probiotic usage, can be viewed as an 'additive approach.'

In addition, the unnecessary exposure of commensals of the gut microbiome to antimicrobials, can lead to establishment of a deep reservoir of genetic determinants within the commensal population, which encode for antimicrobial resistance, and which can subsequently be transferred to gut pathogens.

Antibiotics will always have their place as the primary potent agents with which to treat clinical infectious disease. Where a change must be made however, is not only in the cessation of the prophylactic use of antibiotics and their empitical usage, but also in giving the highest priority to the prevention of disease, and hence avoiding subsequent 'firefighting' with antibiotics. A key underlying strategy here is by way of improving and elevating the immune status of the animal, such that fewer infections can establish and gain traction in the animal.

THE GUT MICROBIOME

The gut microbiome contains trillions of cells, more than the number of somatic cells in the body. This collective gut genome contains 150 times more genes than are present in the entire body. It is now very well established that a healthy gut microbiome is a key driver of the body's overall immune system. Also the enteric mucosal system contains more than a trillion lymphocytes and has a greater concentration of antibodies (including immunoglobulin A,) than any other tissue in the body. Interaction between the enteric epithelial cells and the microbiome is necessary for proper immune development. The mucosal immune system shapes the gut microbiota and vice versa, the gut microbiota influences immune system development. One of the most important molecules involved in 'cross talk' between the immune system and the microbiota is IgA which supports the establishment of a balanced microbiota by regulating its composition, controlling microbial gene expression and increasing microbial diversity, as well as binding and coating specific microbes and antigens in the intestine.

Thus the largest organ of the immune system is, in fact, the gut, making the management of it essential for productivity and health. The enterocytes lining the gut are key cells and respond to metabolites and signalling messengers from the commensals in the lumen to maintain tight junctions. Intestinal inflammatory responses are also modulated by the gut microbiome.

Dysbiosis (i.e., malfunction) of the gut microbiome is caused by an imbalance between the commensals and a preponderance of pathogenic organisms. The commensal microbiome regulates maturation of the mucosal immune system and regulates the general immune system, while the pathogenic microbiome causes immunity dysfunction resulting in disease development. A dysbiotic microbiome will not perform vital functions such as nutrient supply, immune system development, vitamin production and protection from pathogens. In addition to the possible development of resistance (AMR), the unnecessary use of oral broad spectrum antibiotic – by disrupting the ecology and balance of the microbiome – can adversely affect numerous key functions in the gut, not least of which is the immunological. The gastrointestinal commensal population behaves as an anti-infectious barrier by inhibiting adherence of pathogens and their subsequent colonisation of the gut. Commensals also produce bacteriocins and other toxic metabolites inhibiting pathogen growth.

The gut microbiota is a complex, diverse and constantly changing dynamic environment which is made up of bacteria, fungi, viruses, yeasts, protozoa, and archea. In pigs and poultry, it is established that there are more than 900 bacterial species dwelling in the microbiota. High-throughput bacterial and

fungal whole genome sequencing has allowed identification of diverse operational taxonomic groups of micro-organisms in the intestine. It has also allowed identification of new micro-organisms following probiotic administration.

Enteric bacteria constitute a wide range of mostly strictly anaerobic or facultative anaerobic species. In a well-balanced microbial environment, members of the following genera prevail: *Streptococcus, Lactobacillus Bifidobacterium, Enterococcus, Eubacterium, Fusobacterium, Peptostreptococcus, Enterobacter, Bacteroides,* and *Porphyromona*, while the numbers of coliform bacteria *E. coli* and *Clostridium* sp.are lower.

The gut microbiome is not constant; the microbiota composition in all species can change in response to stress, especially weaning. For instance, in pigs at weaning, a microbial shift has been documented to occur as a result of changes to the composition of resident commensal bacteria. This results in a breakdown in barrier function, a decline in transepithelial electrical resistance (TEER), an increase in gut permeability via tight junctions, and a local immunological dysfunction. The result is usually post-weaning diarrhoea.

THE GUT MICROBIOME AND IMMUNITY

The importance and key role of gut commensals for the formation of a fully functional immune system was first studied in germ-free (GF) animals, which are bred and housed in an environment devoid of micro-organisms.

The immune tissues in the intestinal lining and the local immune subsets that are in direct contact with gut microbiota are hugely different in GF animals. Studies have shown functional alterations in immunity and intestinal epithelial cells which express fewer microbe sensing toll like receptors (TLR). Peyers patches, lympoid follicles, T cells, helper cells, antibodies, and immunoglobulins, are all underdeveloped in GF animals, where there is no direct contact with a microbiota. Similarly, the foetal gut is sterile in the uterus and starts to become slowly colonised after birth. This underdevelopment of the neonatal gut microbiome is clearly connected to the lowered immune status of the newborn. The immaturity of the immune system in newborn animals and infants is highlighted by an increased susceptibility to various infectious pathogens, rendering infectious diseases the leading cause for mortality in neonates. Tight junctions are a critical structure in restricting transepithelial permeability. Chemical signals sent from the microbiota, e.g., via the metabolite indole, promote fortification of the epithelial barrier through up-regulation of tight junctions and associated cytoskeletal protein. Pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), sense microbial signals during infection to elicit a protective immune response. TLRs are involved in host defence against pathogens, regulating the commensal population and maintaining tissue integrity. TLRs are of particular importance in shaping the gut microbiota.

Although there is a clear interplay between the gut microbiome and the development of adjacent mucosal immunity in the intestine, additional interactions between the gut microbiome and immune systems in other non-gut organs has now been well documented.

THE CHEMICAL SIGNALLING MECHANISM FROM THE MICROBIOME

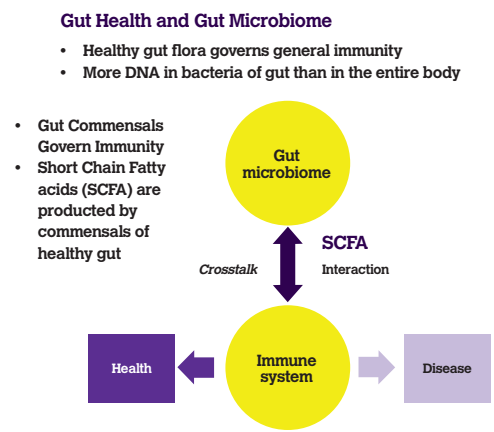
The gut microbiome signalling chemicals and microbiome-associated metabolites translocate from the intestinal lumen to various organs (e.g., the lung) through the circulatory system, and subsequently induce tissue-specific local immune responses. Short Chain Fatty Acids (SCFA) are particularly important here insofar as they are metabolites produced by gut commensals. SCFA acting via G proteins, are a signalling link between the microbiota and the immune system and modulate different aspects of intestinal epithelial cells and leukocyte and cytokine development. SCFA from the gut cells can translocate to the lung and shape the pulmonary immunological landscape, and up-regulate immune function. SCFAs are carboxylic acids with aliphatic tails of 1-6 carbons of which acetate (C2), propionate (C3), and butyrate (C4) are the most abundant produced by anaerobic fermentation of dietary fibres (DF) in the intestine. In addition, the SCFAs, such as acetate, propionate and butyrate, are important metabolites in maintaining intestinal homeostasis. Several studies have shown that SCFA levels are reduced in gut disease. SCFAs are an important fuel for intestinal epithelial cells and are known to strengthen the gut barrier function. Recent findings, however, show that SCFAs, and in particular butyrate, also have important immunomodulatory functions. In the intestinal mucosa, acetate, propionate and butyrate exert beneficial effects over intestinal epithelial cells (IECs) and immune cells through induction of intracellular or extracellular processes. As a result, butyrate maintains and/or increases transepithelial electrical resistance (TEER).

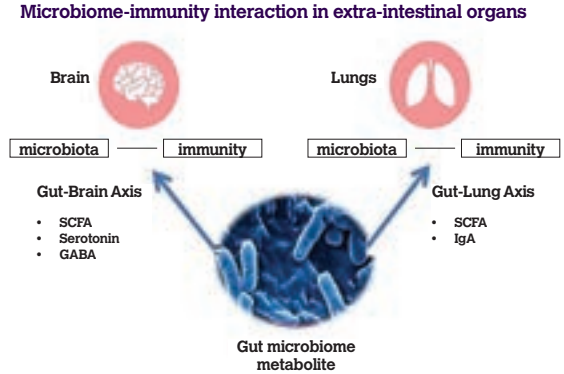
Apart from these physiological functions of SCFAs, they also exert anti-inflammatory effects in the intestinal mucosa. Absorption of SCFAs is facilitated by substrate transporters, and SCFAs may signal through cell surface G-protein coupled receptors. Butyrate influences the activity of histone deacetylases (HDAC), which is responsible for decreasing dendritic cell IL-12 and IL-6 cytokine secretion and allows dendritic cells to promote T cells. Intestinal dendritic cells are located within specific intestinal lymphoid tissues, collectively termed gut associated lymphoid tissues (GALT), or diffusely distributed throughout the intestinal lamina propria. Dendritic cells are the primary cell type involved as 'sensors' of microbial ligands.

In short, the microbiota shape the development of the immune system and the immune system shapes the composition of the microbiota. This mutual interdependence and symbiosis is maintained through life and is the key for a healthy interaction between the microbiota and the immune system. In normal conditions, the immune system controls the growth of commensals and assists in maintaining a stable gut microbiome. In return, a healthy microbiota produces chemical messengers and signalling mechanisms such as SCFA that promote development of immune cells.[1]

CROSS TALK BETWEEN GUT AND LUNGS

A gut-lung axis is now known to exist whereby signalling messengers from the commensals of the healthy gut microbiome (e.g., SCFA) pass to the pulmonary tissues where





they regulate local immunity. IgA is subsequently detected in high concentrations in these tisues. Thus dysbiosis of the gut, which results in lowered SCFA secretion by gut commensals, may predispose to increased susceptibility to respiratory infection. Considerable research in human medicine has definitively documented the manner in which lung health can be dependant on gut health. This is especially true in coeliac disease, crohn's disease, and irritable bowl disease (IBD), where such patients show a disproportionately higher incidence of respiratory ailments. Studies in man have shown that oral antibiotics, which can kill gut micro-organisms, can alter the human immune response to seasonal influenza vaccination. Many studies suggest an important role for the gut-lung axis and highlight the need for intestinal barrier integrity and an optimum commensal-rich microbiome.
Calves suffering from scour are twenty times more susceptible to respiratory infection. This linkage has been seen in field trials with certain probiotics in calves. In these trials, not only was the incidence of scour reduced, but also the incidence of respiratory disease was less in probiotic-treated animals compared to untreated controls.[2,3,4]
'Cross talk' between the gut and other body systems is also known to occur especially with the CNS. Recent evidence has indicated that some neurotransmitters such as dopamine, serotonin and GABA may in fact be synthesised and utilised by the gut microbiota. Accordingly, some human research has focused on mood and behaviour changes possibly resulting from gut dysbiosis. Probiotic-fed piglets have exhibited higher contents of hypothalamic serotonin and dopamine as well as serum γ-aminobutyric acid along with higher colonic concentrations of butyrate and valerate on day 28 of feeding, thus showing both local and central changes in response to probiotics.

PROBIOTICS

The term 'probiotic' comes from two Greek words *(pro and bios)* and it means "for life". The first concept of probiotics was probably suggested in 1907 by Mechnikov, who noted that bacteria may have a beneficial influence on the natural intestinal microflora. The term 'probiotic' was probably invented by Ferdinand Vergin, who in his paper of 1954 entitled *Anti-und Probiotika* compared a harmful effect of antibiotics and other antimicrobial agents on the intestinal microbiota with a beneficial effect ("probiotica") of selected bacteria.
Probiotics are defined as living microbes that, when administered in adequate amounts, confer health benefits on the host. The FDA has required feed manufacturers to use the term "direct-fed microbial" (DFM) instead of "probiotic".
The most frequently used micro-organisms in probiotics are Lactobacillus species (LAB), Enterococcus, Streptococcus, Pediococcus, Bifidobacterium and Saccharomyces.
The addition of a probiotic into the intestinal microbiome will augment the number and range of commensal organisms in the gut population, thus initiating a wide range of beneficial effects, both antimicrobial and immunological.
In a variety of animal models, treatment with probiotics, containing lactic acid bacteria (LAB) have been reported to modulate the gut microbiota, SCFA production and inflammatory responses. They do this by effectively seeding the gut with commensal organisms, restoring microbiome balance and restoring optimum microbiological and immunological flux. Thus, eubiosis rather than dysbiosis is established. Increases in local SCFA production, concurrent with immune homeostasis is connected to probiotic-induced microbial and metabolic changes.
Probiotics create a more favourable gut population due to a shift in the balance of beneficial and harmful organisms. A healthier gut microbiome is associated with enhanced animal performance reflecting more efficient digestion, enhanced immunity and less clinical and sub-clinical disease. The reduction of pathogenic micro-organisms in the gut is also attributable to the production of antimicrobial substances by the probiotic. It is not simply a 'crowding out' effect. For instance, Lactobacillus acidophilus produces bacteriocins, such as nicin, which inhibit cell wall synthesis in pathogens. Pathogenic bacteria communicate cell to cell through the secretion of chemical signals called auto inducers which affect the behaviour of bacteria. This process of bacterial communication called quorum sensing is also used for communication between bacteria and their hosts. Probiotics affect quorum sensing in pathogenic bacteria thus influencing their pathogenicity.

PUTATIVE MECHANISMS OF PROBIOTIC ACTION

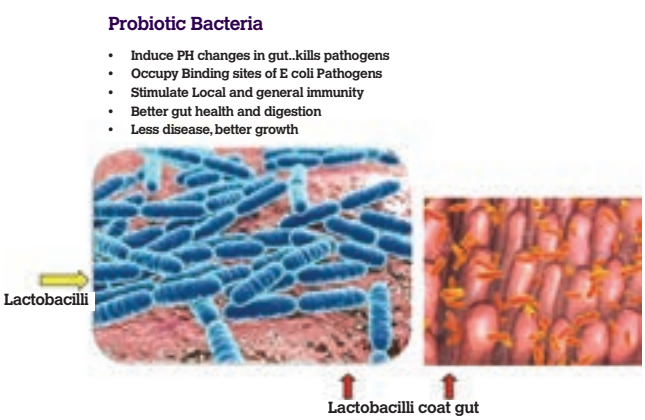
Probable mechanisms of action and effects of probiotics are based on the following:

- Competition between the probiotic organisms Lactobacills/Enterococcus and pathogenic micro-

organisms for binding sites in the intestinal mucosa(competitive exclusion).
- Lactic acid production and lowering of gut PH providing a hostile environment to pathogens.
- Increased nutrient production and availability.
- Inhibition of pathogen growth by production of organic acids and antibiotic-like exotoxins, bacteriocins, H202 and nicin.
- Immunological actions, via immunoglobulin A(IgA) production, augmentation of T cells and killer cells.
- Interaction with protective mechanisms and structures of the enteric mucosa (tight junctions TLRs and dendritic cells) and regeneration of the intestinal mucosa.
- Beneficial alterations in the villus:crypt ratio.
- Regulation of local cytokine production governing inflammation, interleukin production gamma interferon, and TFN.
- Stimulation and activation of the dendritic cells, natural killer cells, macrophages, T and B lymphocytes and neutrophils of the intestines.

Prebiotics are dietary short-chain carbohydrates(oligos accharides,lactulose, inulin), which cannot be digested by animals but are specifically utilisable by intestinal microflora.

They support and potentiate the growth and/or activities of probiotic micro-organisms in the gastrointestinal tract, and act as a microbial 'fertilizer'. Probiotics in combination with prebiotics are referred to as "synbiotics".



BENEFICIAL EFFECTS OF PROBIOTICS IN CALVES AND LAMBS

The calf and lamb's gut is essentially sterile both in utero and in the birth canal, and it becomes colonised after parturition. There is increasing evidence that this complex microbiome plays a crucial role in the development of the mucosal immune system and influences newborn health and strength of the animal. Data is now being generated revealing significant associations between the early microbiome, development of the mucosal immune system, and the growth and health of newborn calves and lambs. The in utero sterile mammalian gastrointestinal tract (GIT) is rapidly colonised by an array of microbiota during and after birth. The microbial colonisation of the neonatal gut is a complex process influenced by a two-way interaction between host and microbes and a variety of external factors, including maternal microbiota, environment, birth process, diet and immune status.

Young animals are born immunologically naïve and with an underdeveloped gut microbiome. Hence they are highly susceptible to infections, especially from microbial pressure in the environment. Probiotics tend to give the greatest benefits in these young, growing animals.

A number of publications are available showing demonstrable productivity effects in calves and lambs. Amongst the various general findings have been:

Calves

- Growth rate benefits, improvement in ADG and weights at weaning, less scour, less respiratory disease.[5,6,7,8,9,10,11]
- Clinical field trials with one particular licensed product (Provita Protect) demonstrated 10 per cent growth rate improvement to weaning, 80 per cent less incidence of scour compared to negative controls 70 per cent less incidence of pneumonia compared to negative controls.[12,13]

Probiotic Effects in Calf Field Trials
(Lactobacillus Acidophilus +Enterococcus Faecium)[12,13]
Provita Protect

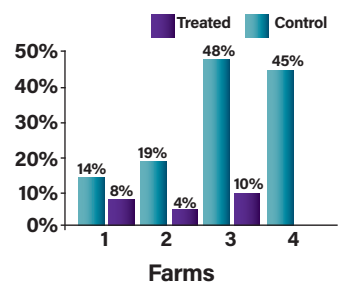


Figure 1: Percentage of calves with scours.

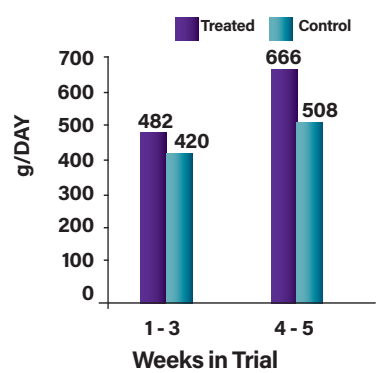
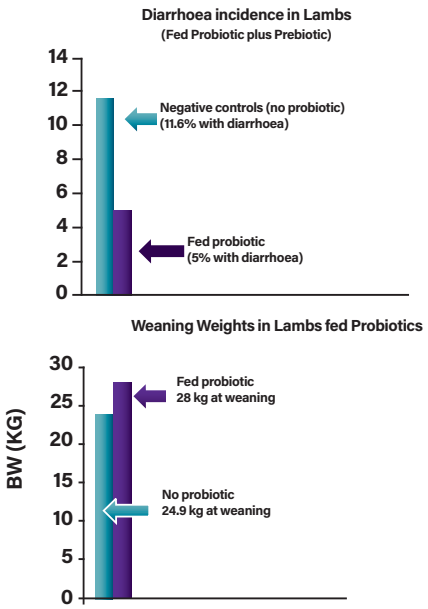


Figure 2: Growth rates.

Lambs

Publications reported improved antibody response, enhanced response to vaccination, increased weight gains and reduced incidence of E coli diarrhoea (watery mouth).[14,15,16] (These trials will be the subject of a second paper)

Lambs: Lactobacillus Casei + Inulin Effects in Lambs[16]



RESURGENCE IN PROBIOTIC INTEREST

The resurgence of interest by veterinary surgeons in proven and effective probiotics as alternatives to antibiotics, has been triggered by a number of recent issues:

(1) the global increase in AMR in food animals and its likely transference to humans;

(2) the worldwide push back on the use of antibiotics in animal husbandry;

(3) new EU regulations on prohibition of prophylactic and metaphylactic use of antibiotics;

(4) the realisation that prevention is better than cure and that avoidance of disease is better than treating it, both financially and on welfare grounds;

(5) the overwhelming recent scientific evidence on the importance of high immunity in contributing to a healthy animal and to the realisation of its genetic potential in growth and productivity; and,

(6) the recent published evidence of the multiple and widespread immunological effects conferred by a healthy gut microbiome.

UNDERESTIMATION OF PROBIOTICS' POTENCY

The term probiotic has confused people, and a number of veterinarians still regard such substances with a degree of scepticism tending to underestimate the scientific potency of proven probiotic products.

Unfortunately, there have been many unproven and substandard 'probiotics' on the veterinary market for quite a number of years . This undesirable situation has arisen because in the early days, probiotics were defined as "live micro-organisms which when administered in adequate amounts could confer a health benefit on the host". In other words, it was a 'possible effect' rather than a 'proven effect.' Furthermore, quality control, characterisation of micro-organism strains, shelf-life of product, storage conditions, number of strains, and regulatory approval of manufacturing premises were not required at that time.

Hence, unproven, unsubstantiated, and unreliable products remained as the majority of probiotic products on the market and were easily accessible by farmers and vets. The lack of standardisation and the lack of proof of any kind of efficacy for the product made it extremely difficult for the veterinarian to distinguish between what was a scientifically evidence-based, effective compound and what was a totally useless product. For this reason only probiotics with evidence-based clinical effects should be used, and this means in most cases that the product should have been approved by a national regulatory authority, in some way, on the basis of clinical efficacy. The overall efficacy of probiotics is influenced by many factors such as quality control in manufacturing, differences in microbial composition (e.g., single or multi-strains), numbers of CFUs (colony forming units), viability, storage conditions, shelf-life, method of administration and dosage, and environmental stress factors, as well as animal's age and health status.

CURRENT RECOMMENDATIONS

Recently, groups of specialist international scientists in the microbiome and probiotic fields, mainly in the human sector, have been convening to review and discuss the above issues regarding the lack of international standardisation of the term 'probiotic.' They have identified the problems and they have set out new criteria in terms of quality controls, proof of efficacy, and when the term 'probiotic product' can be properly used. Their recommendations were published in *Frontiers of Microbiology* (July 2020).[17]

"The term 'probiotic' poses a scientific problem," said the International Scientific Association for Probiotics and Prebiotics (ISAPP). "It does not distinguish between bacterial strains that have a possible health benefit and strains that have a demonstrated health benefit, as shown in studies."

The review calls for probiotics to be supported by at least one clinical trial preferably followed by confirmatory trial(s), and thus evidence-based proof of efficacy.

CONCLUSION

Any new approach to "replace, reduce and rethink" antibiotic usage must be focused on (a), "prevention is better than cure", and the realisation that, in the current AMR climate, 'firefighting' with antibiotics is not a scientifically-sound or appropriate approach, and (b), the realisation that the holistic promotion of gut microbiome health, and the enhanced immunity arising therefrom, is a new and effective way forward. A significant number of international peer reviewed publications in human and veterinary medicine are now supporting and endorsing this concept.

The gut microbiota, the largest symbiotic ecosystem with the host, has been shown to play important roles in maintaining intestinal homeostasis. Dysbiosis of the gut microbiome is caused by the imbalance between the commensal and pathogenic microbiomes. The commensal microbiome regulates the maturation of the mucosal immune system, while the pathogenic microbiome causes immunity dysfunction, resulting in disease development. Therefore, even when used to target susceptible pathogens, treatment with oral broad-spectrum antibiotics that affect a larger proportion

Prevention is better than cure!

Provita Protect

- The only licensed clinically proven calf probiotic on the market
- Scour reduced by 83%
- 31% better daily weight gains
- Better immunity
- Less respiratory disease

**References available on request*

Administer as soon as possible to newborn or bought-in calves. Suitable for use on all dairy and beef calves.

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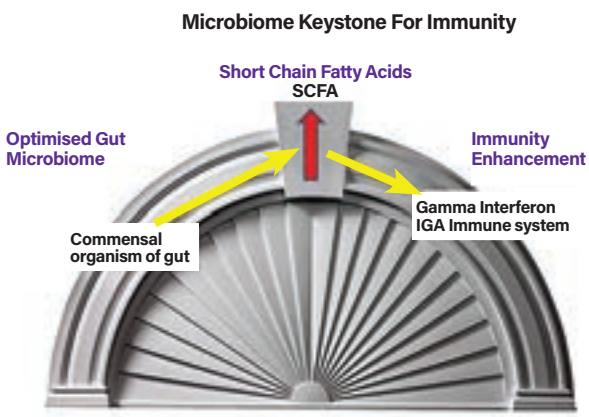
of the gut microbiome and bacterial community may be detrimental to long-term host health via impedance of the immune system or, indeed, AMR development.
Probiotics can play a key role in disease avoidance, but only if given on a prophylactic basis, ideally from birth. Acting as a pre-emptive strike to seed the gut with beneficial commensals, effective probiotics can tilt the balance away from a likely pathogenic infection. Additionally, by augmenting the animal's general immunity it provides not only for gut health, but also protection from infection in other tissues such as the respiratory tract. Probiotics are not therapeutic agents and will not replace therapeutic antibiotics. They can, however, act as suitable non-antibiotic prophylactics to prevent disease when given to neonatal animals, or at periods of stress.



DISCLOSURE: Dr T. Barragry acts as independent advisor to Provita Animal Health Ltd.

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