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Review

The growth potential for dairy probiotics

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ABSTRACT

There has been a rapid rise in global probiotic use, but more recently a drop in dairy applications in Europe, due to regulatory intransigence and rising costs. This review will propose that it is an ideal time for companies to alter their strategic approach, rather than stop the research and development through an inability to obtain health claims. Opportunities exist to expand production of dairy probiotics in the developed and developing world. Data showing effects against diarrhoea, malnutrition, antibiotic side effects, and their potential to affect allergies and mood, and reduce environmental toxin adsorption, provide the scientific basis for shaping a dynamic future for dairy probiotics.

Contents

1. Introduction

The rapid and impressive rise in the sales of probiotic products globally has its roots in the launch of dairy probiotic products, particularly by Yakult, Danone and Valio within the past twenty years. The emphasis placed by these and a few other companies on probiotics that were scientifically and clinically tested, laid the groundwork for future growth, at least until regulators intervened in Europe in the mid-2000s. The support for probiotic food led to the United Nations and World Health Organization assembling an Expert Panel that in 2001 defined probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). This definition has stood the test of time and been widely adopted by scientific, industry and regulatory communities (Hill et al., 2014).

The launch of Activia yoghurt in Canada in 2004 literally transformed the awareness of probiotic concepts. Within one year, an estimated 75% of the 8 million people living in Quebec Province had consumed Activia. This incredible product uptake symbolized an awakening of the public to a concept that made sense health-wise, had a scientific rationale, and had not until then been available. As advocated in 2008 (Reid, 2008), probiotic foods and supplements tested in human studies have since emerged that convey local gut and distant site effects on health (Skokovic-Sunjic, 2014).

While market research companies are in the profit-driven business of projecting the future of probiotic sales, they must rely on scientific trends and progress to understand what technologies and approaches are in the pipeline. This paper will review this

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literature and provide an opinion that probiotic research and development can be the mediators of making health-promoting probiotic dairy products reach consumers worldwide.

2. Probiotics for specific conditions

In almost all cases, dairy probiotics by their nature as a food, are made available to children and adults all year round. This has remained despite attributes of probiotic strains showing some apparently specific characteristics, such as countering seasonal allergies (Koyama et al., 2010; Singh et al., 2013), relieving constipation in adults (Agrawal et al., 2009) not children (Tabbers et al., 2011), reducing drug-associated diarrhoea (de Vrese, Kristen, Rautenberg, Laue, & Schrezenmeir, 2011), reducing the duration of acute respiratory infections in otherwise healthy children and adults (King, Glanville, Sanders, Fitzgerald, & Varley, 2014), reducing anxiety (Messaoudi et al., 2011), and improving immune parameters in HIV patients (Hemsworth, Hekmat, & Reid, 2012). It raises the question of whether someone not suffering from any of these issues need take the probiotic? On the one hand, the clinical effects of these foods are invariably lower than drugs, for example in lowering cholesterol (Fuentes, Lajo, Carrión, & Cuñé, 2013) without the side effects and efficacy levels compared with statins (Tompkins, Schwartzbard, Gianos, Fisher, & Weintraub, 2014), yet on the other hand, probiotics can induce some physiological effects even in a so-called healthy person. For example, four week consumption of *Lactobacillus paracasei* subsp. *paracasei* LC01 in healthy adults resulted in reduced faecal *Escherichia coli* and ammonia, and increases in *Lactobacillus, Bifidobacterium*, and *Roseburia intestinalis* and acetic and butyric acid (Zhang et al., 2013).

The ability of some lactobacilli to produce neurochemicals potentially able to reach the cerebellum via the vagus nerve (Bercik et al., 2011), raises the question of whether such probiotics could benefit people with mood disorders? Certainly animal studies have shown the microbiota gut-brain signalling can be influenced by neural, hormonal, immune and metabolic pathways and potentially affect mood, pain and cognition (Alcock, Maley, & Aktipis, 2014; Borre, Moloney, Clarke, Dinan, & Cryan, 2014). But, too often in science, evidence obtained in mice is presented as meaning the effects will translate in humans. This is a huge assumption where anatomy, mechanisms, diet, housing, and so many other factors are completely different in humans. In one human study that theorized that disruption of the gut epithelial barrier could lead to inflammation and depression, Maes, Kubera, and Leunis (2008) found higher prevalence of antibodies against enterobacterial lipopolysaccharide in patients with major depression than controls. If probiotic organisms can improve gut barrier resistance, as appears to be the case in vitro (Resta-Lenert & Barrett, 2003) and in vivo (Liu et al., 2011), their ingestion might have an effect on mood through at least this mechanism. Certainly, there is precedent for food affecting mood, such as fish and vitamins that increase serotonin, a mood regulator produced in the brain (Lang & Borgwardt, 2013; Tanskanen et al., 2001).

In a unique study using magnetic resonance imaging, four week intake of probiotic yoghurt affected activity of brain regions controlling central processing of emotion and sensation (Tillisch et al., 2013). Numerous other studies are now underway to explore the extent of gut microbiota-brain signalling and the degree to which probiotic foods can influence it (Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2014). Of particular interest will be the effect on healthy controls. Clearly, foods should not adversely affect the mood of people deemed healthy. In such studies, behavioural outcomes need to be measured along with gut barrier resistance and where possible levels of neurochemicals in the bloodstream.

Already, some foods are consumed for specific reasons, such as high fibre bran for constipation. Perhaps products taken only at certain times of the year might also find a niche, for example, probiotics to reduce the duration, symptoms and signs of allergy. Given that claims are permitted on over-the-counter medications, it may be feasible to allow food labels to indicate that consumption of certain probiotic yoghurt prior to and during the allergy season might be beneficial. This is the approach taken by vaccine companies that deliver small amounts of an allergen, such as pollen, pet dander or mould, twice weekly for several months, followed by a less frequent maintenance dose. In a pilot study, we selected probiotic strains based upon their in vitro effects at countering allergic immune parameters (Koyama et al., 2010), and their ability to be formulated into a yoghurt delivery vehicle. One *Bifidobacterium adolescentis* isolate had never been used in yoghurt, but it was added because of its anti-allergic properties. The yoghurt was well tolerated by the subjects, and although it did not have a statistically significant effect on allergy-related quality of life scores, use of antihistamines, or eosinophil cationic protein concentration in nasal lavage in this small study, it did significantly increase serum IL-10 and IL-12 levels during grass allergy season and TGF-b levels during ragweed season. While a larger sample size is needed to prove cause and effect, these small studies can provide useful information to identify potential responders, as long as conclusions are not over-drawn (Hackshaw, 2008).

Further evidence of the potential for probiotics to improve quality of life in allergy patients comes from a double-blind, placebo-controlled, randomized trial in which *L. paracasei* subsp. *paracasei* LP-33 was given to patients with allergic rhinitis who were already being treated with anti-histidine loratadine (Costa et al., 2014). The study showed consistently improved ocular symptoms, but the lack of effect on nasal symptoms, also found in another probiotic study (Ivory et al., 2013), indicates that prevention and treatment of allergy may need different approaches, with perhaps ingested probiotics to target systemic anti-inflammatory effects and delay the occurrence of allergic symptoms (Tamura et al., 2007), and locally-administered probiotics to affect the nasal passage.

A further potential seasonal approach to probiotics comes from studies showing that fermented milk can reduce the risk and duration of the common cold (Guillemard, Tondu, Lacoin, & Schrezenmeir, 2010; Makino et al., 2010). Could such products be sold specifically from November to April in the Northern Hemisphere (http://www.cdc.gov/flu/about/season/flu-season.htm) (Mäkelä et al., 1998) and May to July (http://www.who.int/influenza/surveillance_monitoring/2011_GIP_surveillance_seasonal_review/en/) in the Southern Hemisphere when colds are particularly prevalent? A randomized, double-blinded, placebo-controlled 28-wk intervention study showed that children receiving *Lactobacillus rhamnosus* GG supplemented milk had fewer days with respiratory symptoms than the control group (Kumpu et al., 2013). Interestingly, the probiotic intervention did not reduce the times that viruses infected; however, in another Finnish study using a chewable probiotic tablet with *L. rhamnosus* GG plus *Bifidobacterium animalis* ssp. *lactis* BB-12, there was decreased presence of picornaviruses after 3 months (Lehtoranta et al., 2014). In terms of how a probiotic could interfere with viral infection, a study of 84 men and women engaged in winter endurance-based physical activities, showed that regular ingestion of *Lactobacillus casei* Shirota fermented milk reduced the frequency of upper respiratory tract infections, possibly via increasing salivary IgA levels (Gleeson, Bishop, Oliveira, & Tauler, 2011). Although difficult to prove, the implication is that the antibody in the mouth and throat might neutralize the virus before it reaches the lungs. This is supported by studies showing that probiotic food can enhance mucosal immunity to better eradicate the common

cold virus and relieve its effect on fever, rhinorrhoea, and cough incidence (Groeger et al., 2013; Leyer, Li, Mubasher, Reifer, & Ouwehand, 2009).

If a company simply wanted to re-brand a slightly different version of a popular Product X, by refining the content and labelling as the 'Cold' Brand X, this might be allowed, even if specific disease claims were not permitted. There is certainly a market niche, as shown by a product called Cold-fX derived from the roots of North American ginseng (Panax quinquefolius) that Health Canada's Natural Health Product Directorate approved to "help reduce the frequency, severity and duration of cold and flu symptoms by boosting the immune system". With an over-the-counter cold remedy market worth $8 billion in the USA alone (http://www.ibisworld.com/industry/cough-cold-medicine-manufacturing-otc.html), dairy probiotics that alleviate colds are worthy of pursuit.

The intent of these examples is to suggest that some probiotics could target specific conditions, and still be safe for all who use them only as a food. Yoghurt production lines can easily accommodate different batches, and the distribution networks are in place in developed countries, with relatively short shelf-life turnarounds capable of this targeted use. Current regulatory systems were set up long ago to restrict disease treatment and prevention only to drugs, a dogma that needs a radical overhaul, so it would not be possible for such food products to make specific niche claims in many countries. However, consumer knowledge is acquired from many sources, in particular the internet and medical science experts, and dissemination of clinical studies showing such effects could well drive uptake of the given products. As scientists, it behoves us to educate the public on why scientific studies apply to products.

3. Modification of dairy probiotic formulations

The modification of an existing product is already commonly done through changes to flavour and packaging or addition of fruits, such as prunes for intestinal transit and pomegranate with anti-oxidant or other health benefits. A recent study showed that flavours can alter the genes expressed by probiotic yoghurt organisms (Bisanz, Macklaim, Gloor, & Reid, 2014b), suggesting that a new version of a product that uses a different flavour may have altered probiotic effects (King et al., 2014). For example, if a natural non-flavoured yoghurt improves digestion of lactose, a claim approved by EFSA, could the effect be altered in the presence of certain fruit additives and does this need to be tested? The implications that all food variations need to be re-tested would have significant implications for the dairy industry and regulators.

Of all the benefits accrued by probiotics, arguably their effect on preventing and reducing the duration of diarrhoea, including antibiotic-associated, has been the most documented (Goldenberg et al., 2013; Pattani, Palda, Hwang, & Shah, 2013; Szajewska, Skórka, Ruszczyński, & Gieruszczak-Białek, 2013). Contaminated food alone contributes to over 1.5 billion cases of diarrhoea in children each year, resulting in more than three million premature deaths, according to the World Health Organization (WHO). As dairy foods are less easy to transport between countries where diarrhoea rates are high, this niche has not been targeted by the dairy industry. But, if probiotic dairy products could be dried and packaged or strains delivered to allow household propagation, the probiotic organism's anti-diarrhoeal attributes might reduce morbidity and even mortality associated with diarrhoea. For this to occur, dairy companies would not only have to dry-powder their products, but also develop networks to appropriately retain bacterial viability over time and in different climates. Sachet and stick forms of probiotics are already sold, and large volumes of skim milk are dried and sold as powder, so the creation of dried probiotic and dried fermented milk is feasible. Certainly, the methodologies exist to achieve this (Bauer, Schneider, Behr, Kulozik, & Foerst, 2012; Fonseca, Cenard, & Passot, 2015; Huq, Khan, Khan, Riedl, & Lacroix, 2013).

4. Diet-based products

With tens of billions of dollar spent in the USA alone on weight-loss programs, including drugs and surgeries, and no clinically proven regimen that guarantees success (http://abcnews.go.com/Health/100-million-dieters-20-billion-weight-loss-industry/story?id=16297197), is there an opportunity for fermented dairy probiotic products for obesity? Studies emerging from the Human Microbiome Project have suggested that the gut microbiota of obese individuals differs in composition and/or functionality from that of people who are able to remain lean (Turnbaugh et al., 2009; Vrieze et al., 2010). This implies that obesity is not simply explained by nutritional habits and the reduction of energy expenditure through decreased physical activity, but to a large extent by the organisms in the gut. This has resulted in multiple studies aimed at manipulating these organisms, through intake of prebiotics, probiotics or specific nutrients, or indeed cessation of products such as artificial sweeteners that induce glucose intolerance (Suez et al., 2014).

A particularly interesting intervention was tested in Finland, in which 159 women were randomized and double-blinded to receive *L. rhamnosus* GG or placebo four weeks before expected delivery and postnatally for 6 months (Luoto, Kalliomäki, Laitinen, & Isolauri, 2010). Anthropometric measurements of the children showed that the perinatal probiotic treatment moderated the initial phase of excessive weight gain, especially among children who later became overweight, but it did not affect the second phase of excessive weight gain at age four. This is intriguing as it suggests a potential to program the extent of weight gain.

Some critics have used the weight gain acquired by treatment of livestock as evidence that bacteria, such as *Lactobacillus acidophilus* used in some probiotic products, cause obesity (Million et al., 2012), but there is no such evidence in humans nor any rationale for this to happen (Delzenne & Reid, 2009). In particular, there is no genetic or functional reason for one species of *Lactobacillus* to make people obese, while other species do not. Indeed, ingestion of probiotic yoghurt made using *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 was found to improve total cholesterol and LDL-C concentrations in a randomized double-blind controlled trial of 60 subjects with type 2 diabetes and low-density lipoprotein cholesterol (LDL-C) greater than 2.6 mmol L^{-1} (Ejtahed et al., 2011). Another study showed that probiotic therapy enhanced the effect of metformin in reducing the body mass index (Shavakhi et al., 2013) and one showed it could induce weight loss in women (Sanchez et al., 2014).

While experiments in mice show promise for the use of *Bifidobacterium breve* B-3 in skim milk suppressing the accumulation of body weight and epididymal fat, and improving serum levels of total cholesterol, fasting glucose and insulin (Kondo et al., 2010), it is difficult to correlate this with what happens in humans.

A recent human study suggested that the genetic make-up of an individual can support bacteria, such as Christensenellaceae, and thereby promote a lean figure (Goodrich et al., 2014). This led to the hypothesis that using such a species as a probiotic might alter metabolism and reduce obesity. In a host that is not receptive to this species, perhaps the continual passage of the organism through the gut as a daily probiotic might still confer the desired metabolic outcome. Of course, for such a dairy application, this depends on whether or not these spore-formers could be added safely to milk products, when other spore-forming organisms are infamous for spoilage and causing illness through toxin production (Doyle et al., 2014).

Non-alcoholic fatty liver disease (NAFLD) is associated with obesity, and may be promoted by bacterial endotoxins that induce steatohepatitis. Encouraging animal studies have led to clinical investigation into probiotics for this disease (Eslamparast et al., 2014). A small study suggested that children already suffering from NAFLD might be aided by 8 strain probiotic VSL#3 intake (Alisi et al., 2014). That product is sold as a dried, high concentration sachet rather than in a dairy carrier, but the potential of the approach working in humans is worthy of further pursuit.

The preliminary finding that prebiotic inulin/oligofructose can decrease fat mass in obese women, apparently by increasing gut *Bifidobacterium* and *Faecalibacterium prausnitzii* (Dewulf et al., 2013), is relevant to the dairy field as this mix could be added to fermented milk and delivered as a synbiotic. This would be easier in terms of product development and regulatory approval than having an organism like *F. prausnitzii* which has no history of safe use in humans, scaled-up commercially as a supplement. One recent trial of 97 children aged 7–18 y, who were overweight and obese (BMI >85th percentile) and randomly assigned to receive placebo (maltodextrin) or oligofructose for 12 weeks, did not find loss of weight or body fat (Liber & Szajewska, 2014). Nevertheless, the potential for prebiotics to reduce weight gain and increase weight loss by targeting particular gut microbes is worthy of investigation.

5. Probiotic products sold through social businesses

One of the major road blocks to increasing the global impact of dairy probiotics is a failure to reach billions of people in the developing world. There are several reasons for this, including affordability and availability. Milk production is low in much of Africa and South East Asia, where a large number of people reside and major health issues of malnutrition, stunting, infectious diseases, and maternal and infant mortality are high. In countries like India, where milk is available, and often fermented in households, the protective effect of the yoghurt against chronic diarrhoea depends on the strains used, the dose taken, and the extent of exposure to pathogens. If the Dahi products consumed contain *Lactococcus lactis* ssp. *lactis, Lactococcus lactis* ssp. *cremoris* and *Leuconostoc mesenteroides* ssp. *cremoris*, it may provide some protection (Agarwal & Bhasin, 2002), even though these strains are more noted for conferring flavour than interfering with enteropathogenic bacteria.

Use of highly documented probiotic strains such as *L. rhamnosus* GG and *L. casei* Shirota have been shown to be effective in preventing and controlling diarrhoea in developing countries (Basu, Paul, Ganguly, Chatterjee, & Chandra, 2009; Oberhelman et al., 1998; Sur et al., 2011). But until recently, both strains were protected intellectual property and not available in these countries, and if they were to be sold, the price would likely be well outwith the means of the people who needed them the most. This raises an ethical question of performing such clinical studies then not making an efficacious product available upon its completion.

One solution is to use a generic version of *L. rhamnosus* GG (Kort & Sybesma, 2012). A 1 gram sachet with the probiotic and a *Streptococcus thermophilus* strain is capable of producing 10–100 L fermented milk per batch. The *S. thermophilus* strain replaces the traditional *L. delbreuckii* ssp. *bulgaricus*.

With a break-even point at 10 L, including cost of sachet, milk and labour, there is incentive to produce and market more of this Yoba yoghurt. Even with shipping, the price point of around US$0.65 per sachet is competitive with a single tub of yoghurt sold in retail in Europe. This cost decreases as the number of required sachets increase. Profits of US$0.30 per litre are reached with 100 L of sales, which in rural Uganda is a reasonable return. Two thirds of the yoghurt consumers are regulars and the others incidental. A small portion includes children/orphans, so the social business model is reaching vulnerable subjects and allowing the translation of dairy research to the consumer. It also delivers health and economic benefits to local people. Growth projections are impressive for such a simple set up.

The Yoba probiotic dairy concept was spurred by a humanitarian initiative set up in Tanzania in 2004, in which community kitchens run by women were established to sell *L. rhamnosus* GR-1 supplemented yoghurt. It was assumed that the art of fermentation would be widespread, given the history of use in Africa (Anukam & Reid, 2009; Franz et al., 2014). In fact, this was not the case, and few in the large city of Mwanza, practiced or were aware of how to ferment milk. These Mwanza and Ugandan dairy groups now feeds around 15,000 people each day, illustrating the tremendous potential of social business. There have been numerous positive outcomes including reduction in diarrhoea, rashes and side effects of drug therapy, and in some cases improvements in CD counts in HIV patients (Bisanz et al., 2014a; Dols et al., 2011; Hummelen et al., 2011a, b; Irvine et al., 2010; Irvine, Hummelen, & Hekmat, 2011; Reid, Gough, Enos, & Reid, 2013; Whaling et al., 2011).

It is not traditional practice for dairy companies to target amelioration of diseases, mostly because regulatory agencies set up in the past century state that only drugs can prevent, treat or cure disease. Indeed, recent unfathomable policy decisions in the US and Europe have been backward in their approach to probiotics. The development of new probiotic dairy-based foods to counter infectious and other diseases afflicting billions of people and for whom alternative treatment and prevention regimens are ineffective, too expensive or unavailable, could include helping to reduce side effects of drugs (Zhu et al., 2014), competing with pathogens (Corr, Hill, & Gahan, 2009; Das & Goyal, 2014), enhancing host immune responses to disease (Foligné et al., 2010; Madsen, 2006), or providing microbes and metabolites that restore and retain homoeostasis (Rehman et al., 2012). Although claims may not be permitted on the product labels, the scientific results will be published and through traditional and social media, consumers will hear about the results and create a pull for the products. As long as the messaging is consistent with the quality of the studies, probiotic dairy foods could certainly be promoted more widely to reduce (Adegboye et al., 2012; O'Connor et al., 2014; Sonedstedt et al., 2011) and help treat (Camfield, Owen, Scholey, Pipingas, & Stough, 2011; King et al., 2014) important diseases.

Not all climates are suitable for cows, and therefore milk from goats, camels and buffalo is used by many people. This has been part of traditional food for centuries in areas of the developing world, produced and consumed within households and sold in small quantities in local markets. However, coordination of milk to central processing sites then distribution via cold chains to expansive rural and urban communities either does not occur or is not well-coordinated. With the income per capita too low to support large sales of branded yoghurts and cheeses that are typically sold in Western and Northern countries or South Africa, dairy probiotics must be affordable to all. For example, Zambia has the highest poverty ratio of 86% and is the poorest country in the world, with seven out of ten people living on less than two dollars a day, and an average life expectancy of 51 years (http://www.presscave.com/top-poorest-countries-in-the-world/). If it could better organize milk centralization (Mumba, Pandey, & van der Jagt, 2013) it would have the potential to establish a mechanism to produce and disseminate probiotic dairy foods.

In short, dairy science has made many important advances that have led to foods with highly nutritional, health-conferring properties. However, until people in greatest need of such products gain access, we will have collectively failed in our translational endeavours.

6. Detoxification

The final area of discussion that has great potential for dairy probiotics is in detoxification of environmental pollutants. With many deaths worldwide associated with water, air and soil pollution (Song, Christiani, Wang, & Ren, 2014), and anthropomorphic activity increasing levels of toxins, such as heavy metals and pesticides in the environment (Muir et al., 2009), efforts are needed to prevent adsorption of these compounds into the body of humans and animals. The toxins, adsorbed acutely or via chronic long term exposure, can affect neurological and cognitive development in children and immune and cardiovascular diseases (Karagas et al., 2012).

The detoxification potential for dairy probiotics comes from the finding that lactic acid bacteria have an affinity for many toxic metals and various organic pesticides, making it possible to sequester them prior to their adsorption into the bloodstream and tissues. The most recent and encouraging evidence for this comes from a recent study undertaken in Tanzania. The 30 million people living on Lake Victoria already face major challenges with HIV, malaria, poverty, malnutrition and violence, but added to that they are exposed to high levels of environmental pollutants. Admittedly, the 40 million people living around the Great Lakes of North America are exposed to equally high levels of mercury, while populations in China and India face exposure to a variety of toxic compounds. Unfortunately, while North Americans can choose to avoid eating products of the Great Lakes, people living around Lake Victoria cannot and fish consumption is part of their staple diet. So, although this is far from being an African problem alone, it was decided to study the ability of probiotic yoghurt to sequester heavy metals, following in vitro studies showing this indeed was feasible (Monachese, Burton, & Reid, 2012). The finding of excessive levels of heavy metals in the bloodstream of the Mwanza children compared with Canadian children demonstrated the gravity of the pollution (Bisanz et al., 2014a). The reduction in further adsorption of mercury and arsenic after one month of five days per week consumption of probiotic yoghurt was encouraging, albeit not reaching statistical significance. However, three month treatment of pregnant women did show significantly reduced levels of mercury and arsenic uptake. The mechanism involves the binding of the lactobacilli to the metals, as shown by various microscopy techniques. The research has also identified mercury reductase genes as a potential means to further reduce mercury adsorption.

This is a new area of application for dairy science, but other examples exist in countering environmental pollutants. One study, notably from China, has shown that fermented skimmed milk accelerated degradation of organophosphorus pesticides chlorpyrifos, diazinon, fenitrothion, malathion and methyl parathion, in part due to phosphatase production by the lactic acid bacteria (Zhang, Xu, Liu, & Zhao, 2014). This is interesting not only because of the high use of pesticides in China, but because the dairy market, especially milk and yoghurt, has grown substantially. With pastures at a premium, oil prices fluctuating, and cost of feed escalating, new models are needed to help dairy markets continue to grow.

Another example is the ability of lactobacilli to bind to mycotoxins produced by *Aspergillus* species in pre- or post-harvest cereals and milk (Hamidi et al., 2013). When tested in humans, a statistically significant decrease in urinary concentration occurred when probiotics were consumed twice daily for five weeks (El-Nezami et al., 2006). A staggering 4.5 billion people living in developing countries are estimated to be chronically exposed to largely uncontrolled amounts of the toxins (Williams et al., 2004). This exposure significant increases the risk of liver cancer. With in vitro data showing that *L. rhamnosus* GG reduces aflatoxin B1 transport, metabolism and toxicity to intestinal cells (Gratz et al., 2007), the Yoba generic version of this strain in probiotic yoghurt could prove effective in high aflatoxin contaminated areas of east Africa.

7. What represents sufficient evidence?

The concept of probiotics is not without its critics, and the insufficiency of clinical trial data, or failings of some study designs are often cited as evidence that probiotics have not been proven to impact human health. Such a topic may warrant a separate review, but some points are worthy of note. It is unreasonable to suggest that naturally occurring food grade bacteria delivered as supplement or food need to be tested to the same extent as novel synthetic chemicals being considered as drugs. Nevertheless, the FDA itself states in relation to drug approvals, "in some cases, FDA has relied on pertinent information from other adequate and well-controlled studies of a drug, such as studies of other doses and regimens, of other dosage forms, in other stages of disease, in other populations, and of different endpoints, to support a single adequate and well-controlled study demonstrating effectiveness of a new use", and "In other cases, FDA has relied on only a single adequate and well-controlled efficacy study to support approval" (FDA, 1998). Still, many drugs are approved with insufficient evidence, as shown in a European study of 200 unique new medicines, 161 standard and 39 orphan medicines, approved by the European Medicines Agency, where the median total number of patients studied before approval was 1708 for the former and 438 for the latter, insufficient to evaluate safety and long-term efficacy (Duijnhoven et al., 2013). In short, there is no magic bullet for proving efficacy, and all products developed for human use should be continually monitored and tested. Probiotics are, for the most part, not even attempting to claim efficacy, so the fact that clinical trials (many of which have been cited here) have been performed in randomized, placebo-controlled fashion with hundreds of subjects, is laudable. Critics may not be convinced even by the meta-analyses cited here and elsewhere. Guidelines that provide a third party assessment of the volume of documentation and levels of evidence at least help guide consumers in choosing which probiotic may benefit them (Skokovic-Sunjic, 2014). In truth, patients have long relied on healthcare professionals providing them with such advice on drugs, and that process is far from perfect (Velo & Minuz, 2009).

The most important point is that all products using the term probiotic should have undergone scientific rigor, be produced to the highest standards possible particularly if used for vulnerable subjects, and be tested in humans as best as is practical and possible. Studies of 20–500 subjects should not be discouraged simply because of size; all studies can reveal useful information. But overstating effectiveness does not advance the credibility of the field.

8. Conclusions

Many challenges face the dairy industry worldwide. Traditionally strong markets in Europe have been adversely affected by politically motivated legislation, rising production costs and trends towards organic and 'natural' foods. Nevertheless, the globalization of food distribution provides new opportunities, not the least of which are markets in the Middle East and Asia where per capita income can support the purchase of high end dairy products, and there is a custom of eating fermented milk products (Chilton, Burton, & Reid, 2015). The benefits that lactic acid bacteria have provided throughout human evolution need to be re-invigorated through research, development and application. The probiotic market is witnessing unprecedented growth, and the application to dairy products has the potential to improve the lives of millions of people worldwide.

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