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Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion effects and health properties and consequences for extrapolation of farm animal results to humans

M. Bernardeau[1] and J.-P. Vernoux[2]

1) *DuPont Industrial Biosciences—Danisco Animal Nutrition, Marlborough, UK* and 2) *Unité de Recherche Aliments Bioprocédés Toxicologie Environnements EA 4651, Université de Caen Basse-Normandie, Caen, France*

Abstract

For many years, microbial adjuncts have been used to supplement the diets of farm animals and humans. They have evolved since the 1990s to become known as probiotics, i.e. functional food with health benefits. After the discovery of a possible link between manipulation of gut microflora in mice and obesity, a focus on the use of these beneficial microbes that act on gut microflora in animal farming was undertaken and compared with the use of probiotics for food. Beneficial microbes added to feed are classified at a regulatory level as zootechnical additives, in the category of gut flora stabilizers for healthy animals and are regulated up to strain level in Europe. Intended effects are improvement of performance characteristics, which are strain dependent and growth enhancement is not a prerequisite. In fact, increase of body weight is not commonly reported and its frequency is around 25% of the published data examined here. However, when a Body Weight Gain (BWG) was found in the literature, it was generally moderate (lower than or close to 10%) and this over a reduced period of their short industrial life. When it was higher than 10%, it could be explained as an indirect consequence of the alleviation of the weight losses linked to stressful intensive rearing conditions or health deficiency. However, regulations on feed do not consider the health effects because animals are supposed to be healthy, so there is no requirement for reporting healthy effects in the standard European dossier. The regulations governing the addition of beneficial microorganisms to food are less stringent than for feed and no dossier is required if a species has a Qualified Presumption of Safety status. The microbial strain marketed is not submitted to any regulation and its properties (including BWG) do not need to be studied. Only claims for functional or healthy properties are regulated and again growth effect is not included. However, recent studies on probiotic effects showed that BWG could also be observed in humans, or not, according to species and strains. Determining the significance of farm animal results for extrapolation to humans, especially regarding body weight improvement, was not easy because they do not use the same microbial strains nor always the same species. Furthermore, the framework for the management of microbials added to feed or to food differ, especially with regard to goal, timescale and lifestyle. So no one can exclude the possibility that beneficial microorganisms having probiotic effects may have long-term effects in humans that cannot be seen to date in animals, where short-term use is the rule. A possible link to obesity cannot be excluded in relation to timescale, species and strain specificity. To conclude, beneficial microorganisms added in feed are key factors stringently regulated for short-term improvement of zootechnical performances in animals and their use does not entirely parallel that of human probiotics. So extrapolation of farm animal results to humans is biased and not sufficient to be conclusive regarding the existence or not of a link between probiotics and obesity. From a toxicological and nutritional point of view and considering recent findings on a link between antibiotic use in early life and excessive risk of becoming overweight, one suggestion is to study the at-risk population in Europe, pregnant women and their babies before and after birth and during early childhood, in an epidemiological long-term cohort survey.

Keywords: Benefits, feed, food, probiotic, regulation

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Corresponding author: J.-P. Vernoux, Unité de Recherche Aliments Bioprocédés Toxicologie Environnements (UR ABTE) EA 4651, Université de Caen Basse-Normandie, Bd Maréchal Juin, F 14032 Caen, France

E-mail: jean-paul.vernoux@unicaen.fr

Presentation and Comparison of Probiotics use in the Feed/food Chain

Probiotics are defined as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host' [1]. They cover a wide range of living microorganisms with supposed positive effects on gut flora and producing a large number of substances (defined or not) supporting many different effects which are, for the time being, far from proven. For example, many specific health claims proposed by industrial partners for food probiotics failed recently to be validated by the European Food Safety Agency commission because of insufficient scientific or clinical evidence [2]. To date, probiotics are classified in the category of functional products [3] and considered to be beneficial microorganisms [4]. However, regulatory texts do not mention the word probiotic anywhere, and refer to beneficial microorganisms without any other details. This situation differs from that of antibiotics, which also act on gut microbiota and are used in animal husbandry. Antibiotics are stable chemical substances, have a well-defined specificity, and were recognized and named as growth promoters in regulatory texts until the publication of the regulation (EC) 1831/2003 [5] in which they were banned.

Beneficial microbes are feed additives in Europe [6] and are called Direct Fed Microbials in other world regions. In animal husbandry, they were initially used, in the twentieth century, to reduce intestinal colonization by *Salmonella* in chickens, to increase the feed utilization efficiency and reduce diarrhoea in pigs, and to increase milk production and decrease diarrhoea in cattle. Their beneficial effects were complementary with those of the antibiotics used simultaneously as growth promoters at low doses in animal diets [7]. This growth promoter effect observed with antibiotics was supposed to be related to gut health stabilization [7] and it was a proof that manipulation of animals' microflora through diet was efficient in enhancing animals' productivity. So when antibiotics were banned from the feed market in 2006 [8], interest in beneficial microbes having potentially similar effects increased and their regulation was updated in 2003 [5,9].

In human food, beneficial microorganisms are mainly present in fermented items [10] or as ingredients or processing aids and are known to have a long history of use, especially through the use of fermented milk products [6]. The use of probiotics as ingredients or functional foods conferring a health benefit has gained scientific attention since the 1980s, first in Japan then in Europe, and it has mushroomed since 2000 [4]. However, a recent suggestion hypothesized that a widespread and haphazard ingestion of microorganisms may promote obesity in humans by altering the intestinal flora balance [11,12]. This remains controversial and probiotic specialists reacted quickly to this bombshell [13,14], especially the well-known and recognized International Scientific Association of Probiotics and Prebiotics, which was founded in 2000 [15]. However, the discovery of a link between the manipulation of gut microflora and obesity, as demonstrated in mice for certain bacterial species [16], lends credibility to this hypothesis [17]. In the present themed review, we underline some important differences between microbial feed additives and probiotics for food, taking into account species and strains requirements resulting from the regulations in force and the main literature dealing with effects in farm animals or humans, especially body weight gain and health properties. An evaluation of the significance of extrapolation of data from animal studies to humans is presented.

Comparison of Species and Strains Requirements in Feed/food

Both bacteria and yeast are used as microbial feed additives. Around 20 microbial feed additives are authorized in the European Union [9]. Depending on the animal species, ruminant, pig or poultry, specific microorganisms are preferred, i.e. yeast (especially *Saccharomyces cerevisiae*) plays a major role in ruminants, whereas *Bacillus* spp., *Enterococcus* spp. and *Lactobacillus* spp. are more likely to be efficient in pigs and poultry [18,19]. Different strains belonging to similar species have different properties and so effects/benefits can be different from one strain to another within the same species [20].

According to regulation 1831/2003/EC [5], in force at the time of writing, microorganisms are authorized as 'zootechnical additives' for feed. A zootechnical additive is 'any additive used to affect favourably the performance of animals in good health or used to affect favourably the environment'. This group includes, among others, gut flora stabilizers, a category that includes microorganisms. Applications for approval must follow guidelines to establish a relevant dossier [21]. Approval is granted for a strain or a mix for which molecular characterization and identification at species and strain levels are needed. Zootechnical performance for at least one characteristic must be demonstrated to obtain an authorization for a target animal species only and more specifically for a category of age for this species (i.e. weaning piglets, post-weaning piglets, fattening pigs and sows) but growth enhancement is not a prerequisite (the category 'Growth Enhancers' was deleted in EEC regulation 1831/2003 [5]). In fact these additives 'must affect favourably animal production, performance or welfare by affecting the gastrointestinal flora or digestibility of feedingstuffs' as cited in the article 5(3) of regulation 1831/2003/EC [5]. Performance characteristics include feed efficiency through improvement of feed conversion ratio, average daily weight gain through improvement of body weight gain (BWG), milk or egg production, carcass composition or herd performance [22]. A scientific dossier for the approval process is needed for each marketed product.

To put microorganisms on the feed market or to improve their efficiency, much of the current research focuses on the choice and the properties of a suitable strain. It is important (but not required for the European dossier) to understand the mode of action of probiotics in the gut. This makes it possible to achieve a better level of control and to define appropriate dosages for a specific target. For the choice of strains, basic *in vitro* prerequisite criteria, related to identification, enumeration [23,24], safety, gut survival and colonization ability, as well as other criteria related to technological process and probiosis, are necessary [25]. It is worth mentioning that in general only a few strains have the right basic profile [26] and only 1‰ become a marketable strain. This improves the accumulation of knowledge and reinforces the quality and traceability of those beneficial microbes and the supposed reproducibility of a given effect at a strain level along with appropriate dose administration. However, in spite of all of these accurate precautions of selection, the results of probiotic supplementation are still dependent on numerous known and unknown parameters: doses, compatibility with other additives present in the diet, type of feeding, technology used to formulate the diet (pelleting or not), type of animal target, quality of hygiene in the herd and environment [27].

Regulations governing the addition of beneficial microorganisms with probiotic effects to food are less stringent than for feed; the simple fact of belonging to a species with a known safe history of use with a Qualified Presumption of Safety status is enough and no dossier is required. Furthermore, there is no regulation at the strain level for human probiotics used as ingredients. Within a species with Qualified Presumption of Safety status, many different strains can be used whatever their phenotypic and genotypic specificities although important genomic intraspecies variations have been identified, for example for lactobacilli (*Lactobacillus acidophilus* [28], *Lactobacillus casei* [29], *Lactobacillus plantarum* [30], *Lactobacillus salivarius* [31]). Those genomic strain-specific diversities can provide important phenotypic differences associated with a range of effects from adverse [32] to positive [20]. These intra-species specificities are illustrated by the following examples: adhesion and mucus-binding properties in *Lactobacillus reuteri* strains [33]; mechanisms of protection of transepithelial barrier function in *L. salivarius* strains (bacteriocin and hydrogen peroxide production) [34]; and enzymatic production such as that of α-glucosidase activity in lactic acid bacteria, a trait considered negative for diabetic and obese humans [35]. So knowledge of strain-specific properties is lacking in food use and this is the main critical point for a targeted use. This explains recent failures in validation of the specific health claims proposed by industrial partners for these food probiotics by the European Food Safety Agency commission because of insufficient scientific or clinical evidence [2,20].

Comparison of Occurrence of BWG in Animals and Humans

As a result of the increase in livestock production, modern methods of massive livestock rearing (i.e. intensive industrial production) have generated many animal stresses, especially density stress. As a result, digestive diseases have increased and animal performances have been negatively affected [18]. Microbial adjuncts were used as diet supplements to counteract those performance losses. Simon *et al.* [36] who compiled data from the literature published between 1973 and 2000, found that BWG and feed conversion ratio improvements were rare for this period of time with such supplements. Efficiency of these microbial adjuncts can also be deduced from the review of Bernardeau *et al.* [4]: from 46 published animal trials where *Lactobacillus* strains were used, only ten trials showed a significant BWG. In another review [6] where different species and strains of probiotics were tested in different animal trials, eight of the 33 referenced trials presented a significant BWG. Other data gave similar results [37]. So increase of body weight is not commonly reported and its frequency is around 25% of published data examined here. However, when BWG was found in the literature (Table 1) it was generally lower than or close to 10% (Table 1) as previously reported [38,39]. Recently [40], BWG was shown to be species dependent and some species had negligible effects on weight or reduced it; however, some species can improve weight gain by >10%, especially *Lactobacillus ingluviei* in chickens and ducks [41], and by >20% in fish or shrimp (Table 1) over a reduced period of their short industrial lifespan as defined in the regulatory guidelines [42]. This BWG is mainly a protein anabolism inducing lean meat formation rather than a fatty weight gain [14] and is consequently compliant with consumer and public policy objectives. For example, it has been demonstrated that beneficial microbes fed to weaner and grower–finisher pigs provide significantly higher proportions of carcasses classified in the top two categories of the SEUROP scale (S, superior,

TABLE 1. Recent examples of beneficial microbes used in animal husbandry and having growth promotion effects

Animal target	Strains	Inclusion dose	Significant improvement of growth parameters compared with negative control	Hypothetical mode of action proposed by authors [reference]
Poultry production				
Broilers	Processed at Low/high drying temperature *Lactobacillus acidophilus* KNU 31 *Bacillus subtilis* KNU 42 *Saccharomyces cerevisiae* KNU 55 *Aspergillus oryzae* KNU 48	LT/HT $4.0 \times 10^8/1.0 \times 10^2$ $4.8 \times 10^9/2.0 \times 10^4$ $1.0 \times 10^4/1.2 \times 10^2$ $4.3 \times 10^7/1.0 \times 10^3$	BWG at day 42 LT/HT +9.0%/ +7.5%	Higher gains and better feed conversion ratios may be due to the greater crude protein retention [64]
Broilers	*Clostridium butyricum*	3×10^7 CFU *C. butyricum*/kg of diet	+3.7% BWG at day 42	[65]
Broilers	*B. subtilis* LS 1–2	0.45% in diet	+8.35% BWG at day 35	Greater nutrient retention and improvement of gut health [66]
Broilers	*L. acidophilus* *B. subtilis* *S. cerevisiae*	10^7 CFU multi-microbe probiotic/kg of diet, 10^8 CFU multi-microbe probiotic/kg of diet, 10^9 CFU multi-microbe probiotic/kg of diet	+4.87% BWG at day 35 +8.28% BWG at day 35 +8.53% BWG at day 35	Greater apparent digestibility of nutrients and improvement of gut health [67]
Broilers	*Lactobacillus ingluviei* CIP 102980	4×10^{10} *Lactobacillus* spp. per animal inoculated: once, or twice	+10.2% BWG +13.5% BWG	[41]
Ducks	*L. ingluviei* CIP 102980	4×10^{10} *Lactobacillus* spp. per animal inoculated: once, or twice	+7.7% BWG +14% BWG	[41]
Pig production				
Pigs	*Propionibacterium freudenreichii* ssp. *shermanii* CIRM-BIA129	Pigs gavaged daily with 2×10^{10} CFU of *P. freudenreichii* CIRM-BIA129 for 2 weeks	+10% BWG at day 14	Production of vitamins, modulation of the intestinal microbiota or anti-inflammatory properties [68]
Growing-finishing pigs	Two strains of *Bacillus licheniformis* and one strain of *B. subtilis*	1.47×10^8 CFU of *Bacillus* per g of supplement added at 0.05% in diet	G:F increased in finisher phase and in the overall growing-finishing period.	Production of extracellular degrading enzymes, better nutrient digestion and utilization of feed; modulation of immunity [69]
Ruminant production				
Preweaning calves	*B. subtilis* natto	Daily dose of 1×10^{10} CFU of *B. subtilis natto*	FE—15.5% ADG + 12.9% Advance the weaning age of the calves—7.3 days	[70]
Male buffalo calves *Bubalus bubalis*	*L. acidophilus* *S. cerevisiae*	*L. acidophilus* and *S. cerevisiae* at the dose of 1×10^9 and 3×10^9 and CFU/flask/kg	FBW + 4%	Beneficial influence on rumen fermentation [71]
Aquaculture				
Fish *Epinephelus coioides.*	*S. cerevisiae* P13	10^3, 10^5 and 10^7 CFU kg/diet	Variations compared with control groups: PWG (10^3): +6.66% PWG (10^5): +19.9% PWG (10^7): +27%	Colonize the intestines, improve FE and growth rate, induce upregulation of innate cellular and humoral immune responses, increase the resistance to challenge by *Streptococcus* sp. and iridovirus [72]
Fish *Oreochromis niloticus*	*Rhodopseudomonas palustris* G06	Added to water at final concentration of 1×10^7 CFU/mL every 2 days	Higher final weight, BWG +23.37% at day 40 DWG + 22.64% SGR + 12.28%	Enhance immune and health status, thereby improving growth performance [73]
Shrimp *Litopenaeus vannamei*	*B. subtilis* strains, L10 and G1	Two different doses 10^5 and 10^8 CFU/g feed until the end of the experiment (8 weeks)	Dose 10^5 CFU/g feed FW: +36.14% BWG: +45.60% Dose 10^8 CFU/g feed FW: +38.95% BWG: +53.29%	Competitive exclusion, creation of a hostile environment for pathogen colonization; reduction of *Vibrio* spp. populations in the gastrointestinal tract; induction of digestive enzymes stimulate the natural digestive enzyme activity of the host; improve appetite [74]

Growth parameter abbreviations: ADG, average daily gain; BWG, body weight gain; DWG, daily weight gain; F, feed; FE, feed efficiency; FBW, final body weight; G, gain; LT/HT, low drying temperature/high drying temperature; PWG, percentage of weight gain = [100 × (final body weight − initial body weight) (initial body weight)$^{-1}$]; SGR, specific growth rate.

and E, excellent: lean meat >55%) giving an additional benefit to the farmer [43]. The mode of action of beneficial microbes is still hypothetical (Table 1) and has been reviewed in animals [44].

Human probiotics that promote health benefits have received a boost through increasing consumer demand for such products [45]. As a consequence, there has been extensive research, opening up doors for the use of probiotics and in various areas [46]. Million *et al.* [40] were the first to identify growth effects of lactobacilli in humans, including infants <2 years old, lean adults and overweight/obese adults, and highlighted that moderate BWG in humans is *Lactobacillus* species-dependent.

Health Effects and Relation to Weight Gain in Animals and Humans

Several studies (Table 1) have highlighted the health value of lactobacilli in pigs, poultry, cattle, fish and other animals [4].

Those benefits may be a result of the active metabolites synthesized by probiotic microorganisms, such as organic acids, hydrogen peroxide, bacteriocins or bacteriocin-like substances and cell-wall components, and imply their common adjuvant properties in relation to immunity [47]. Probiotic supplementation is recommended for the treatment or prevention of a range of stress conditions and diseases in a number of species submitted to intensive rearing and promiscuity [18]. In fact, growth enhancement effects are more likely in situations involving a stress of some kind, as found on real farms rather than in university-based trials, assuming that health effects and zootechnical effects are closely related [4]. In animals, ingestion of a supplemented diet containing selected microorganisms presented as probiotics makes it possible to counteract some of the negative effects of stress [48,49] and leads to a compensatory BWG. For example, in mice it was reported that with a conventional diet, there were no significant differences between the mice receiving and those not receiving the probiotic supplementation, in contrast to mice fed a sub-optimal diet (i.e. having a nutritional stress) for which BWG was around 30% with probiotic supplementation, although the nutritional value of these microorganisms alone is negligible [50].

The use of live bacterial cultures in the animal industry, whether to improve resistance to specific pathogens or to non-specifically enhance animal health, improves production parameters [51]. This health approach, although not taken into consideration in regulation (which targets 'animals already in good health', as indicated above), is reminiscent of that described for food probiotics for which functionality is associated with health benefits. So, modification of feed additive regulation to include health effects (as Welfare additives or Product quality additives) was proposed by the FEEDAP Panel [52] at the European level, but this approach has not been successful to date and some hurdles were recently identified regarding use of probiotics in feed/food [2]. In fact, regulations on feed do not consider health effects, even if supported by consistent scientific knowledge proving health benefits (which implicitly justify their use), because animals are supposed to be healthy, so such regulations are not required for a European dossier.

In food, the microbial strain used is not submitted to any regulation and so its properties (including BWG) do not need to be studied. However, as intended uses are for functional or healthy properties only probiotic claims are recognized and regulated [53] and again effect on body weight is not included. In humans, recent papers have discussed the limits of current studies related to the significance of the link between probiotics and weight gain or obesity [54–56].

Significance of Animal Results for Extrapolation to Humans

To assess the significance of animal results for extrapolation to humans we chose to see if there were any data where the same probiotic strain was used in both animals and humans. To our knowledge, no beneficial microbes commercialized for feed/food use in Europe or other world regions have been simultaneously used both in animal husbandry and in humans, probably because of the risk of confusing the marketed image. We therefore focused on published data related to research (Table 2). Effects concern mainly gut stabilization and immunity, and with a few exceptions they are not identical for animals or humans, as reported in the corresponding data (Table 2). Extrapolation of farm animal results to humans is therefore biased and not sufficient to make a conclusive argument for the existence or not of a link between probiotics and obesity because we do not have any sustained history of use, nor any data regarding the same strain applied both to animals and humans.

To find an explanation for the aforementioned differences, we addressed framework characteristics between microbials for feed and probiotics for food because they differ greatly, especially with regard to their conditions of use and their goals (Table 3). In the timescale, long-term effects are also expected from food probiotics, whereas feed microbial additives, to be economically useful, must produce a quick response. For example, typical industrial lifespans are 42–80 days for a broiler chicken, around 120–200 days for shrimps, 6 months for pigs, 18–24 months for fish, a few months for calves and a few years for beef cattle. This duration is <5% of the entire life expectancies of the corresponding animal species, which are generally >10 years except for fish and shrimp (5–7 years). As regards specific use in humans, safety assessment should integrate long-term effects and consider possible chronic effects, which were not deducible in humans on the basis of this short-term use in animals. The stress effects linked to lifestyle could also have a different incidence in humans, when compared with animals because rearing in animals is exclusively density dependent (intensive breeding) in contrast to humans evolving in a less constrained environment.

Discussion and Conclusion

The main points identified here concerning the action of beneficial microorganisms added to feed/food on farm animals and humans are (i) regulations for species and strain requirements is not parallel in feed/food; (ii) BWG is moderate in animals and humans and not frequent; (iii) health effects in

TABLE 2. Effects of identical beneficial strains studied for potential use in animal production and for human consumption (adapted from Bernardeau and Vernoux, [6] and updated)

Strains	Human applications	Animal applications
Bacillus cereus ATCC 14893 = IP 5832	Bactisubtil®: probiotic medication used since 1955 under taxonomic label *Bacillus subtilis* IP 5832 to treat diarrhoea, but is in fact *Bacillus cereus* ATCC 14893, used in the 20th century as a feed additive under the commercial name 'Paciflor' (Prodeta, Vannes, France) [75]	Paciflor: strain widely used before 2003 as feed additive in animal nutrition (Paciflor™; Prodeta) [75] Sow and litter: administration benefits the end of pregnancy and lactation period of sows and improves the survival and growth of their offspring during suckling and the flat-deck period [76].
Escherichia coli Nissle 1917 (EcN)	Adult patients with inflammatory bowel disease: a systematic review [77] Premature infants: significantly stimulates specific humoral and cellular responses and simultaneously induces non-specific natural immunity [78] Children, infants and toddlers: exerts a strong immunomodulatory effect, stops acute diarrhoea [79] Human patients: strain frequently used for the treatment of gastrointestinal complaints and is well tolerated [80]; significant improvement of irritable bowel syndrome-related constipation [81]; overview of the mechanisms of action and clinical studies related to Mutaflor (EcN) [82]	Pigs: prevents acute secretory diarrhoea in pigs infected with enterotoxigenic *E. coli* Abbotstown [83]. Piglets: EcN colonizes the intestine and persists in conventionally reared piglets for at least 4 weeks upon oral administration [84] Swine: partial establishment of the strain in swine herds [85]; EcN is partially established in swine herds in Germany with individual variability [85] Adult healthy female pigs[a]: EcN administration not sufficient for stable colonization of porcine gut but induced significant changes in the enterobacterial microbiota [86] Calf: clear beneficial effect on the prophylaxis and treatment of neonatal calf diarrhoea [87]
Lactobacillus rhamnosus GG	LGG®, Gefilus® and Gefilac® are trademarks of Valio Ltd. Together with other branded products containing the same organisms; they are sold in 35 countries around the world (http://ammattilaiset.valio.fi/portal/page/portal/valiocom) Prenatal: prenatal administration failed to modulate diversity of early infant gut microbiota despite promoting a beneficial bifidobacteria profile [88] Fetal status: maternal probiotic supplementation significantly modulated the expression of Toll-like receptor-related genes both in the placenta and in the fetal gut. Microbial contact *in utero* is associated with changes in fetal intestinal innate immune gene expression profile [89] Extremely low-birth-weight infants (ELBW): improves growth velocity in ELBW infants, but no improvement in the % of infants with growth delay at 34 weeks postmenstrual age, no adverse events [90] Infants from 0 to 6 months: children grew better [59] Indian children with acute watery diarrhoea (AWD): effective to decrease the frequency and duration of diarrhoea and reduction in hospital stay of AWD patients [91]	Piglets: does not prevent or reduce the detrimental effect of the *E. coli* F4 infection on the growth performance and health status of weaned piglet [92]; effective in ameliorating diarrhoea in post-weaning piglets induced by *E. coli* K88, possibly via modulation of intestinal microflora, enhancement of intestinal antibody defence, and regulation of production of systemic inflammatory cytokines [93] Horses and foals: absence of negative side effects [94] Rainbow trout: enhancement of immune parameters [95] Nile tilapia: important regulator of gut associated immune systems [96] Fish: suppression of fish pathogen growth [97] Dogs: reduction of carriage of *Clostridium perfringens* [98]; absence of negative side effects and colonization at 10^{11} CFU/g [94]; unsuccessful to treat tylosin-responsive diarrhoea [99] Chickens: binds to aflatoxin AFB1 *in vivo* [100] Calves: absence of negative side effects (as no D-lactate is produced), survive intestinal transit in young calf [101]
Lactobacillus reuteri MM53 = ATCC 55730	Children: abundant colonization of the gastrointestinal tract of infants from birth up to 1 year of age [102]; reduction of the number of febrile episodes and episodes of gastrointestinal infection, doctors' visits and antibiotic use in children [103] Adults: significantly stimulates immune system in the mucosa by increasing the number of B lymphocytes in the duodenum and the number of T lymphocytes in the ileum [104]; reduce gastrointestinal illness and infections and the incidence and the severity of diarrhoea of different origins [105] Hospitalized adults: decreases antibiotic-associated diarrhoea and safely tolerated when administered twice daily for 4 weeks [106]	Weaning pigs: produces the same body weight gain as sub-therapeutic levels of antibiotic [107]; daily fluctuation in specific bands of *L. reuteri* MM53 revealing an antagonistic relationship between MM53 and another indigenous *Lactobacillus* assemblage [108] Chick infection model: antimicrobial activity and immunomodulation *in vitro*, which were confirmed *in vivo* by the use of animal models [109]
Lactobacillus casei Shirota	Dairy fermented milk helping to maintain a healthy balance of the intestinal flora and good for digestion (http://www.yakult.com.my/html/faqs.html) Smokers: higher increase in cytotoxic activity and $CD16^+$ cell numbers in comparison to the placebo intake group [110] Patients and cancer: review suggests that this specific strain may help the reinforcement of defence system against cancer by modulating innate immune functions [111]	Pigs: acts on gastrointestinal tract transit, stimulate colonic fermentation and indigenous *Lactobacillus* [112–114] Rabbit: protects against Shiga toxin-producing *E. coli* O157:H7 [115] Fish: sensitive to fish bile [97]

[a]Small adult pigs were used as experimental omnivore models to study human gastrointestinal functions.

animals and humans were observed and could explained the compensatory BWG; (iv) significance of results in animals is biased and not realistic for extrapolation to humans because of different framework characteristics especially goal, timescale, lifestyle and species and strain specificities.

Data on farm animals are not sufficient for the purpose of developing a conclusive argument for or against the existence of a link between probiotics and obesity and no one can exclude the possibility that beneficial microorganisms with probiotic effects may have long-term effects in humans that cannot be seen in animals, where short-term use is the rule.

The development of food probiotics occurred at the end of the second millennium and the epidemic of obesity in Europe has been on the increase since the beginning of the third millennium [57] so there is a need for research to discover whether a link exists. A better way to investigate this question would be to work directly on human cohorts. From a toxicological and nutritional point of view, and considering recent findings on antibiotic use in early life and an associated and inadmissibly high risk of becoming overweight [58], the at-risk population to be taken into consideration should be pregnant women and their babies before and after birth and during childhood. Zwiauer [59] reported that there are limited data from well-designed controlled studies on the growth of infants fed formulas supplemented with probiotics. The study also pointed out that these widely used formulas presented no indication of negative influence on infant growth in accordance with previous studies, which also reported a growth effect

TABLE 3. Differences between framework characteristics of microbial feed additives and probiotics for food when applied for animal or human nutrition (adapted from [6])

Ingestion (duration)	Human nutrition All life	Animal nutrition <5% of life expectancy
Goal	Long-term effects, health benefits, well being	Quick response, zootechnical performance
Safety	No acute or chronic oral toxicity	No acute oral toxicity
Effectiveness	Difficult to assess	Easy to assess
Intake characteristics	Wet media	Dry media
Nutritional supply	Daily diversity of offer	Daily uniformity of offer
Matrix	Included in small portion of food Often via fermented milk	As additive in mixed feed Plant material (feed matrix)
Frequency of intake	Once per day or more	10–20 times per day
Microorganisms (most frequent)	*Lactobacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp.	*Enterococcus* spp., *Bacillus* spp., *Saccharomyces cerevisiae*
Number of probiotic strains	Mono- or multi-strain products.	First generation: multi-strain Second generation: mono-strain
Process	Organoleptic characteristics (taste, aspect) important Non-drastic dairy technology	Organoleptic characteristics not important Pelleting process very drastic
Storage	+4°C	Room temperature
Natural habitat	Digestive tract, milk products	Digestive tract, soil, fruits
Regulation	None if history of use in humans	Severe
Lifestyle	Variable	Density stressed under farming and sedentary conditions

[60,61]. In France, only appropriate epidemiological surveys, such as the current French national cohort study Elfe [62], reinforced by the current French national study Epifane [63] could provide answers to this crucial question of the origins of obesity. Meanwhile, promotion of 'pharmacovigilance' regarding probiotics applied for human use, especially by at-risk populations as proposed by Bernardeau and Vernoux [4], is highly recommended and should be investigated by scientists as well as industrialists.

Transparency Declaration

The authors declare no conflicts of interest related to this manuscript. Comments in this review are the author's independent opinions.

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