Prevention of intestinal disorders in European pig production after 2006 (ban of antibiotics as growth promoters), with a specific focus on probiotics

Dr. BERNARDEAU M.[1,2], Pr. VERNOUX J.P.[2]
[1], SORBIAL, route de Spay F-72700 Allonnes, mbernardeau@sorbial.fr
[2], Research Unit Microorganisms of Milk & Food Interest, University of Caen Basse-Normandie, F-14032 Caen cedex, jean-paul.vernoux@unicaen.fr

Introduction

In developed countries, modern methods of livestock rearing (i.e. intensive industrial production) may generate animal stress and create a breeding ground for emerging diseases with consequences for public health. As regards the animal itself, factors such as feed, environment, housing conditions and therapeutic treatments have added to the stresses and stressful situations that can affect the animal's resistance, immunity and zootechnical performance (Figure 1). Further, for reasons of profitability and efficiency, modern methods of livestock rearing very often involve the very early weaning (21 days for pigs) and quick absence of the mother. This deprives young animals of the opportunity to acquire a protective microflora from the mother, so they are left unprotected against colonization by pathogenic micro-organisms. One major consequence of increasingly intensive production and the concentration of animals in small spaces is the increasing incidence of animal infections, many of which pose a threat to human health (zoonotic diseases), and declining animal immunity. Although trade is expanding much faster than production, it is constantly under threat from disease outbreaks and this puts increasing pressure on veterinary services to improve their management of transboundary diseases.

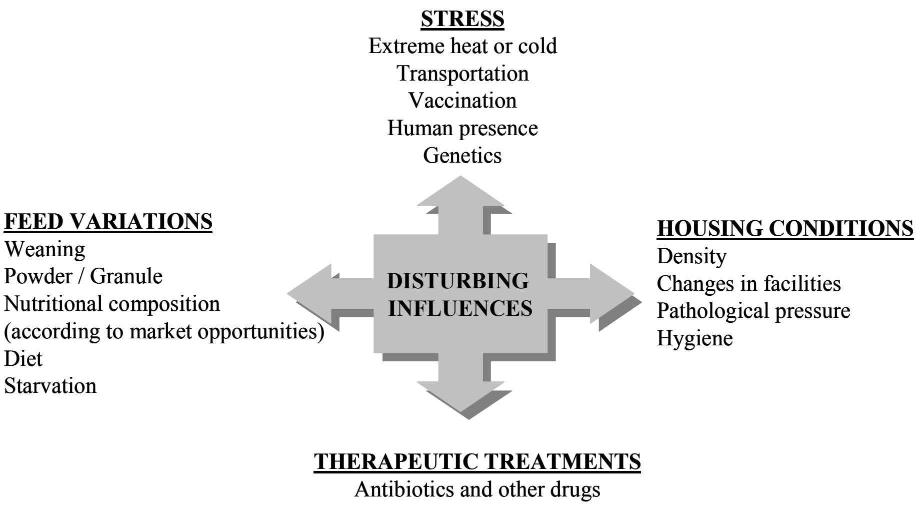

Figure 1: Consequences of intensive farming on animals

All these disturbing influences affect the intestinal microflora balance and, indirectly, animal health. The commensal bacteria are hard pressed to play their leading antagonistic or barrier role in reducing the proliferation of pathogenic opportunistic and resistant bacteria and preventing colonization. As a result, digestive diseases increase and animal performance (body weight gain, feed conversion) is affected. This both decreases the herd's profitability and creates a pathogenic pressure that threatens public health (Figure 2).

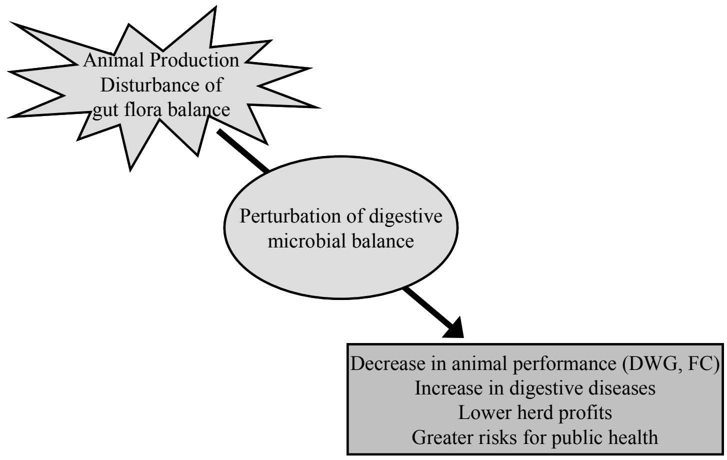

Figure 2: Impact of rearing practises on animal performance and animal production (DWG-daily Weight Gain; FC-Feed Conversion)

It is an important goal for animal production to achieve a microflora balance that will be optimum for animal health and performances. Animals' microflora can be manipulated through diet by means of feed additives. Feed additives offer an opportunity to reduce these problems and to improve animal productivity and health. The incorporation of antibiotic type growth promoters (AGP) in animal feed mixtures has made it possible to improve animal health conditions while increasing rearing intensity. This has successfully lowered food production costs and so benefited the consumer.

History of use of Antibiotic Growth Promoters in Europe:

Since the discovery and development of the first antibiotics prior to the Second World War, these drugs have played an important role in curing disease in humans and animals. From 1946 experiments showed that low, subtherapeutic levels of antibiotics could increase feed efficiency and growth in food animals, and the addition of various antibiotics to feed for livestock was initiated. Because prevention of disease transmission and enhancement of growth and feed efficiency are critical in modern animal husbandry, there has been widespread incorporation of antibiotics into animal feeds in many countries.

Information describing exactly how much antimicrobials go into animal feed for growth promoter uses and how much are being used to treat diseases in animals is hard to come by. One reason is that manufacturers have not been forthcoming about such figures. According to a study by the European Federation of Animal Health (FEDESA), in 1999 farm animals consumed 4700 tonnes (35%) of all the antibiotics administered in the European Union, while humans consumed 8500 tonnes (65%). Of the antibiotics that were given to animals, 3 900 tonnes (or 29% of the total usage) were administered to help sick animals recover from disease, while 786 tonnes (or 6% of the total usage) were given to farm animals in their feed as growth promoters. The survey estimates that the amount of antibiotics used as growth promoters fell by 50% since 1997, when animals consumed around 1600 tonnes as feed additives (http://ec.europa.eu/health/ph/others/antimicrob_resist/am_02_en.pdf).

However, these antibiotics have had an impact on safety, causing two major problems (Dibner & Richards, 2005). One is that chemical residues from such chemicals or drugs may be found in the end product as foreign substances that should have no place in the food chain. The other is that the molecules used in veterinary medicine were the same as those used in human medicine (Teuber, 2001). The use of Antibiotic Growth Promoters –AGP- was soon incriminated as contributing to selection pressure, resistance reservoirs and transmission routes (Gersema, & Helling, 1986). Major debates regarding the use of AGP have occurred during the last 35 years in the EU regarding the potential risk related to the antibiotic resistance in humans. From the Swann Committee report of 1969 concerns were raised on the potential problems due to the use of human antibiotics as AGP in food producing animals and transference of resistance to humans. This correlation has never been clearly demonstrated or supported by evidence and currently, the potential for agricultural antibiotics to contribute to the development of antibiotic-resistant bacteria of human concern is the subject of intense debate and research in the world.

However, European Community was the first to review the technical information and changing social attitudes to the use of additives in animal feed. Early in 1974, the antibacterials used in humans, such as tetracycline, lincosamides and others were eliminated from the European approved product list. A new wave of discussion started during the late 1990s, driven by the Nordic countries, on the risk of development of vancomycin resistant strains due to the use of avoparcin in animals. In spite of the fact that the Scientific Committee of Animal Nutrition (SCAN) did not support the evidence of this link, the EU has introduced legislation, which effectively bans from 1997 the use of avoparcin and ardacin (Commission directive 97/6/EC of the 30 January) in animal feed. The same process occurred later for four other antibiotics (zinc bacitracin, tylosin phosphate, virginiamycin and spiramycin). Only four antibiotics remained (avilamycin used for piglets, pigs, flavophospholipol used for rabbits laying hens, chickens for fattening, turkeys, piglets, pigs, calves and cattle for fattening, monensin sodium used for cattle for fattening, salinomycin used for piglets, pigs for fattening, chickens for fattening and turkeys) each of which was not a member of any other class. Each possesses a narrow spectrum of activity with no cross resistance with products used in humans or for veterinary therapy. From January 1, 2006 the use of these was no longer permitted. All these measures were in line with the Commission's overall Strategy to combat the threat to human, animal and plant health posed by anti-microbial resistance.

However, in January 2006, member states of the EU and different livestock sectors were at varying stages of preparation for the ban. In terms of livestock groups, the European swine industry was believed to be the most impacted, followed by the poultry industry. Southern Europe was likely to feel the effects of the ban most strongly because (1) many farmers in these member states were lack exposure to the range of alternative feed additives available and the benefits they offer, (2) some of them were resistant to the changes taking place and were reluctant to explore new product opportunities. On the other hand, Scandinavia was expected to be the least affected, since it was well prepared with bans already in place in Sweden and Denmark. Overall, the withdrawal of antibiotic growth promoters has impacted countries in the EU in much the same way as it affected Sweden and Denmark - with reduced animal growth rates and decreased feed conversion efficiency.

Additionally, the cessation of antibiotic use in livestock was proposed to be accompanied by innovations that improve animal health and make the use of antibiotics unnecessary. In June 2005, delegates from European countries met in Brussels to discuss methods of raising animals without antibiotics. The group's suggestions included changing farming practices, such as weaning pigs later to give them a chance to develop sturdier immune systems; developing new veterinary drugs; and feeding animals dietary supplements. One barrier to progress evoked was the lack of knowledge of basic animal digestive physiology, particularly bacterial colonization of animal digestive tracts. Further research on this topic would generate new alternatives to antibiotics for European and other livestock producers.

Emerging Opportunities for Alternative Feed Additives

As the ban was drawing closer to full implementation, livestock producers were looking to identify new ways that stimulate production among animals and deliver the same benefits provided by AGP. This created a significant opportunity for alternative feed additives. Moreover, it was supposed that countries outside the EU that import animal products such as chicken and pork meat would found it difficult to continue doing so. This opened up new opportunities for those within the European animal feed industry to supply such countries with alternative feed additives.

Several different feed additives emerged or "re-emerged" as potential alternatives. However, the general belief is that there is no direct replacement available for antibiotic growth promoters and further research needs to be done on alternative products to prove their effectiveness. Furthermore, AGP were considered both inexpensive and effective whereas alternative products are said to be often expensive in comparison. Although alternative feed supplements may compensate to some extent for the reduction or elimination of antibiotics in feeds, some changes in pig husbandry practices may also be important. A global view of the different feed alternatives to AGP in pig husbandry is proposed in Table 1.

Table 1 : Global view of non-exhaustive alternatives feed ingredients and husbandry practices to antibiotic growth promoters in pig (adapted from Doyle, 2001).

Alternatives Feed Ingredients	Alternatives Husbandry Practices
Probiotics	Good hygiene
Enzymes	- efficient cleaning methods
Immune modulators	- effective sanitizers
Organic acids	- appropriate ventilation rate
Minerals	- appropriate environmental temperature
Vitamins	- stocking rates appropriate for size of the farm
Conjugated linoleic acid	Weaning
Phospholipids	- segregating
Amino acids	- all in/all out
Carnithine	Feed practices
Carbohydrates (Polysaccharifdes: fiber)	Biotechnology / Genetics
Herbs	

History of use of probiotics in animal nutrition

Interestingly, the use of antibiotics as feed supplements and the demonstration that they could improve growth and feed conversion ratios stimulated research into their mode of action and coincidentally into the composition of the gastrointestinal microflora. This increased interest in the gut microflora showed that it comprised both beneficial and potentially harmful micro-organisms, and that these could compete with each other. This gave rise to the popularity of the probiotics, defined as live cultures of microbes—often lactic acid bacteria but also some other species—which are fed to animals to improve health and growth by altering intestinal microbial balance. Some authors also consider extracts of these cultures, for example isolated yeast cell walls, to be probiotics even though they do not contain living cells (Doyle, 2001).

Probiotics have been marketed by suppliers and used on farms since the 1960s. This use was encouraged by the Swann Committee in 1969 which recommended that antibiotics in animal feeds be restricted to those that are not used therapeutically. This created a lack which probiotics began to fill. In the 1970s a great deal of effort was expanded trying to improve the growth and health status of farm animal by modifying the indigenous intestinal flora using live microbial adjuncts as probiotics. In 1970, Europe introduced strict regulations on the use of feed additives. These regulations did not cover probiotics, since they were regarded as natural substances. Over the next 20 years, between 1970 and 1990, probiotics were widely commercialized for use in farm animals, with claims of better growth, feed efficiency and health benefits (less diarrhoea, lower mortality rates etc.). Throughout this period, with no regulatory framework, numerous probiotic products were marketed with claims supported by no scientific evidence.

The literature published between 1973 and 2000 illustrate the rather rough-and-ready approach of that period; statistical improvements in weight gain, feed conversion ratios or other zootechnical parameters are rare. In Simon et al's review of pigs feed supplementation, 93% and 95% of the results were non-significant on daily weight gain and feed conversion ratio respectively, including 27% with negative effects (Simon et al., 2001). However, they pointed out that in some herds, long-term diarrhoea had been successfully treated with probiotic preparations. Differences in the effect on weight gain and feed conversion may depend on the species or strain of bacteria used, the dosage in the feed and the development stage at which they are administered (mother sow, suckling piglet, weanling). Studies with probiotics have been difficult to assess because many of the earlier studies were not statistically analysed, experimental protocols were not clearly defined, micro-organisms were not clearly identified, and the viability of the organisms ingested was not verified (Stavric & Kornegay, 1995). In many cases the animals' environmental and stress status was neither considered nor reported (Patterson & Burkholder, 2003) although it has some importance, since animals under stress and/or suboptimal rearing conditions respond more strongly to probiotics (Jørgensen, 1988). This led the scientific community to view "probiotics" with some scepticism (Bernardeau & Vernoux, 2008). Professional veterinarians, nutritionists and farmers also expressed some antipathy towards the probiotic concept and the probiotics manufacturers, with the result that the supply of probiotic products on the European market declined in the early 1990s. This is why we have called the probiotic products used up to 1993 "**the first generation of probiotics for animals**", characterised by the assumption of efficacy, the lack of regulations at the time and, in most cases, the lack of scientific evidence for their efficacy and mode of action (Bernardeau & Vernoux, 2008).

Between 1980 and 2000 there was a series of crises in Europe with bovine spongiform encephalitis, dioxin contamination etc. As a result, the whole product chain is being overhauled, starting with a regulatory framework for livestock rearing practices designed to protect the entire food chain. Loss of confidence by the European public also led to a complete overhaul of the European Union's food safety system and policies. In January 2002 the European Food Safety Authority (EFSA) was set up. EFSA was established to ensure a high level of consumer protection and so restore and maintain consumer confidence. One of EFSA's first activities was to reshuffle Dir. 70/524/EC governing the use of feed additives. This led in 2003 to the new official text, Reg. 1831/2003/EC. The feed additives regulations have also become increasingly stringent and micro-organism safety assessment requirements have been made tougher. Although introduction of regulations has placed a heavy burden on companies in terms of financial and human resources, it has definitively put an end to the rough-and-ready approach of the first generation of probiotics use in farm animals.

Despite Metchnikoff's pioneering work in the early 20th century, attention has only focused on human applications of probiotics since the 1980s and progressed over last 10 years, leading to a remarkable improvement of the knowledge about the role of intestinal micro-organisms (Caramia, 2004). Over the same period there has been a revival of scientific interest in the use of probiotic micro-organism for livestock. These recent studies are well-designed, randomized, placebo-controlled double-blind studies conducted to high scientific standards. They focus on understanding the modes of action of probiotics in gut, aiming for better control and appropriate dosage for specific target animals. Research has also revealed new health opportunities for probiotics strains. Today, probiotic supplementation is recommended for the treatment or prevention of a range of stress conditions and diseases in a number of species (Table 2). "Probiotic therapy" is also becoming increasingly popular in veterinary medicine, particularly for pets. Recent scientific articles have highlighted the therapeutic potential of specific strains.

Table 2: Overview of sanitary effects of probiotics recently demonstrated in pig husbandry (adapted from Bernardeau et al., 2006)

Animal	Probiotic strain	Comments	References
Pigs	Strain of *Lactobacillus casei*	Increases BWG of PRRS virus infected pigs. Does not affect immune response	Kritas & Morrison, 2007
Weanling pigs	Strain of *Saccharomyces cerevisiae*	Increases BWG related to increase in FC Does not modify bacterial populations, yeast numbers or VFA concentration	Li et al., 2006
Sow	*Escherichia coli* Nissle 1917	Long-term colonization and transmission in a swine herd, individual persistence, colonization	Kleta et al., 2006
Piglets	*Enterococcus faecium* EK13	Decreases *E. coli* counts in faecal samples. Decreases cholesterol and increases concentrations of total serum protein, calcium, haemoglobin, haematocrit, red blood cell count and phagocytic activity index of leukocytes. Does not influence BWG or total counts of bacteria	Stompfova et al., 2006
Pigs	*Lb. farciminis MA27/6R and Lb. rhamnosus MA27/6B*	In vitro inhibition of the viability, spreading and adhesion of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*, the causative agents of respectively the Swine Dysentery and the Porcine Intestinal Spirocheaetosis	Bernardeau 2006
Pigs	*Lb. casei (Shirota)*	Survives GIT transit; effect on fermentation in the large intestine;	Ohashi et al., 2004
Pigs	Strains of *Lb. johnsonii; Lb. pentosus*	Reduces *Salmonella* load	Casey et al., 2004
Pigs	*Lb. salivarius* DPC6005, *Lb. pentosus* DPC6004,	Decreases Enterobacteriaceae counts in faecal samples	Gardiner et al., 2004
Minipigs	*Lb.* species	Decreases faecal enzyme activity of beta-glucuronidase and azoreductase (markers for procarcinogenic activity)	Haberer et al., 2003

Microbial adjuncts for feed are now subject to regulation **1831/2003/EC**, which contains two major changes compared to the previous one. These concern (1) the depletion of national approval prior to European evaluation; (2) a reshuffling of the additives categories: 5 main groups in place of the original 15, according to the properties of the product (technological, sensory, nutritional, zootechnical or coccidiostatic and histomonostatic). Most micro-organisms belong to the "zootechnical additives" group, which includes growth enhancers, digestibility enhancers and gut flora stabilisers. Applications for approval must follow guidelines, and approval is granted for one animal species only and more specifically for a category (Becquet, 2003). Assessing a feed additive under Reg. **1831/2003/EC** is a complex process, requiring a comprehensive, multidisciplinary approach to assess all aspects relevant to the use of the substance in question. Compounds intended for deliberate addition to or use in animal feed must have proven efficacy and must be safe for animals and consumers at the intended dose levels (Mantovani et al., 2006). Their ecotoxicity and their safety for users/workers must be assessed. Massive use of feed additives in intensively farmed animals may lead to a significant environmental exposure through animal excreta. As regards micro-organisms, concerns about residues are unlikely; safety evaluation is focused on such issues as production of toxins, residual pathogenicity and induction of cross-resistance; sensitization of workers to microbial protein products might also deserve attention. Thus feed additives now require careful evaluation based on up-to-date scientific information, in order to establish their efficacy and safety in modern livestock farming (Mantovani et al., 2006). The requirements for putting together application dossiers have forced the manufacturers to advance scientific knowledge of their probiotic strains and probiotic products. This includes basic science and technological, safety and efficacy data. Published data are derived from research conducted to high scientific standards with well-designed, randomized, placebo-controlled, double-blind experiments. Much of the research focuses on understanding the modes of action of probiotics in the gut, to achieve better control and appropriate dosages for specific target animals. In this way there has been a revival of interest in what we call **"the second generation of probiotics in animal husbandry",** which are safer, more efficient and more transparent than those of the first generation.

Prevention of intestinal diseases in pig production illustrated by *Lb. rhamnosus* 3698 and *Lb. farciminis* 3699 probiotic potential

Since 1996, we have been studied the probiotic potential of two lactobacilli strains: *Lb. rhamnosus* CNCM-I-3698 (MA27/6B) and *Lb. farciminis* CNCM –I-3699 (MA27/6R) from a French company named Sorbial. Based on *in vitro* and *in vivo* studies, we established an important body of knowledge (Figure 3). Specific studies focused on their possible applications as feed supplement in animal nutrition, especially in pig production for the prevention of intestinal disorders.

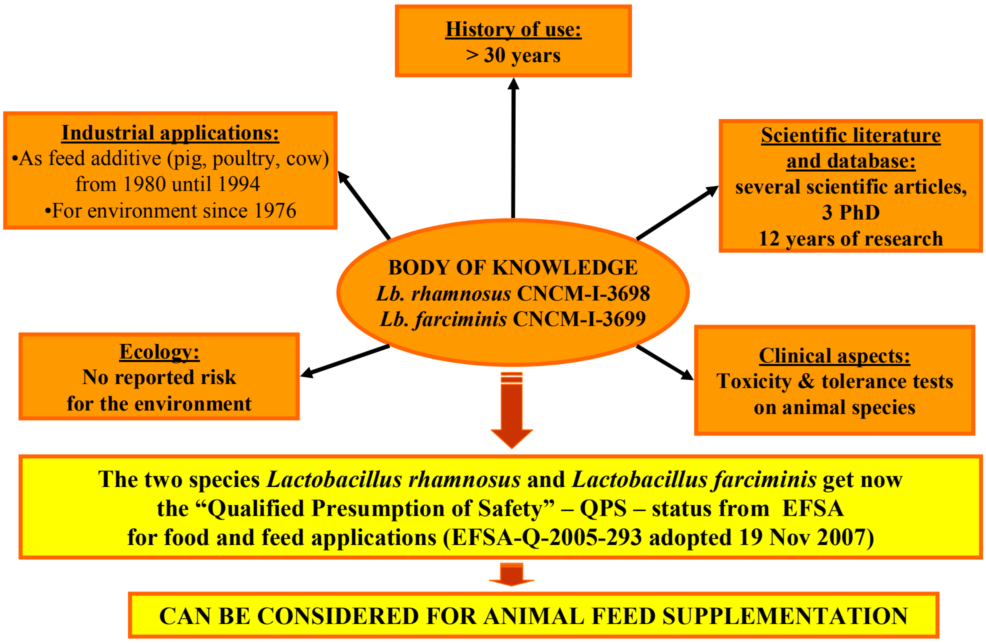

Figure 3 : Body of knowledge of probiotic strains *Lb. rhamnosus* CNCM-I-3698 and *Lb. farciminis* CNCM-I-3699.

Both strains were characterized phenotypically and genotypically (including PFGE profiles). They do not carry acquired resistance genes to the current antibiotics. They are not toxic for insect larvae (*Galleria mellonella*), mice or weanling piglets (daily high dose). They resist to gastrointestinal tract conditions *in vitro* and *in vivo* (mice and human). They have *in vitro* functional probiotic properties: adhesion to a gastro-intestinal cellular model (Bernardeau et al., 2001a); and synthesis of anti-microbial substances active against tested pathogens: *E. coli, Salmonella, Clostridia, Listeria, Helicobacter,* (Bernardeau et al., 2001b) *Campylobacter* and *Brachyspira* (Bernardeau 2006). Considering the interactions with pathogenic agents, three mechanisms of actions have been identified *in vitro* as possible ways to prevent diseases: (1) inhibition of the pathogen's viability due to lactic acid and bacteriocin-like substance(s) and inhibition its growth due to morphological changes; (2) limitation of its spreading due to aggregation with lactobacilli metabolites, coaggregation with lactobacilli cells and its loss of motility; (3) and prevention of its adhesion due to lactobacilli barrier effect. *In vivo*, feed supplemented with those 2 lactobacilli can enhance zootechnical performances of piglets and pigs, improve the performances of the sows, decrease diarrhoea in the herd and increase the profit of the breeder.

Conclusion

Europe has taken the lead on reducing antibiotic use in livestock and ensuring the safety of the food supply for its citizens. This forced the different actors to innovate and to applied properly feed and practices alternatives in order to keep the herd safe and economically profitable. Microbial adjuncts named probiotics seem now to be relevant alternatives as they succeeded in getting more confident with users. This came from the important demand formulated by the European legislation in place in terms of characterization, safety and efficacy data and from the scientific advances in the area of digestive microbiota. More than zootechnical additives, probiotics now appear to be a viable disease control strategy and so should help secure international market access for animal products (Chinabut & Puttinaowarat, 2005).

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