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Consequences of probiotics release in the intestine of animals

J.F. Guillot
Université de Tours-IUT, 29, rue du Pont-Volant, 37082 Tours Cedex 2, France

SUMMARY – Probiotics are live microorganisms that, when administered through the digestive tract, have a positive impact on the host's health. Microorganisms used in animal feed are mainly bacterial strains belonging to different genera, e.g. *Lactobacillus, Enterococcus, Pediococcus* and *Bacillus*. Other probiotics are microscopic fungi, including *Saccharomyces* yeasts. Some probiotic microorganisms are normal residents in the digestive tract, while others are not. A probiotic dose of 10^6 to 10^7 CFU/g of feed administered continuously is necessary to obtain a balance between probiotic microorganisms and bacteria of the resident microflora in the gut. Different mechanisms of probiotic action have been suggested, but most are only hypothetical. The positive effect can result either from a direct nutritional effect of the probiotic, or a "health" effect, with probiotics acting as bioregulators of the intestinal microflora and reinforcing the host's natural defences. Probiotics generally show low efficiency under controlled experimental conditions, but under animal husbrandy conditions, the activity of some probiotics seems better when the gut microflora is unbalanced. In 1994, EC directive guidelines for the assessment of additives in animal nutrition were modified to include probiotics and enzymes (EC directive 94/40). Action mechanisms of all probiotics should be fully defined in order to improve their future use.

Key words: Animal feeding, microorganisms, probiotics, growth, feed conversion efficiency, animal health.

RESUME – *"Conséquences de l'administration de probiotiques dans l'intestin des animaux". Les probiotiques sont des micro-organismes vivants qui, administrés par voie digestive, ont un effet positif sur la santé de l'hôte. Les micro-organismes utilisés en alimentation animale sont essentiellement des souches bactériennes appartenant à différents genres, par exemple* Lactobacillus, Enterococcus, Pediococcus *et* Bacillus. *D'autres probiotiques sont des champignons microscopiques incluant des levures du genre* Saccharomyces. *Certains micro-organismes probiotiques font partie des hôtes normaux du tube digestif, alors que d'autres n'en sont pas. Une dose de probiotique de 10^6 à 10^7 par gramme d'aliment, administrée en continu, est nécessaire pour obtenir dans le tube digestif un équilibre entre le micro-organisme probiotique et les bactéries résidentes de la microflore. Différents mécanismes d'action des probiotiques ont été proposés mais la plupart sont hypothétiques. L'effet favorable peut résulter soit d'un effet nutritionnel direct, soit d'un effet sanitaire, les probiotiques agissant alors comme des biorégulateurs de la microflore intestinale en renforçant les défenses naturelles de l'hôte. Dans des conditions expérimentales contrôlées les probiotiques montrent généralement une faible efficacité mais dans les conditions d'élevage l'activité de certains probiotiques semble meilleure, en particulier lorsque l'équilibre de la microflore digestive est perturbé. En 1994, la directive européenne réglementant l'autorisation des additifs en alimentation animale a été modifiée pour inclure les probiotiques et les enzymes (directive 94/40/EC). L'avenir des probiotiques est lié à une meilleure connaissance de leurs modes d'action et à la mise au point de souches microbiennes plus efficaces.*

Mots-clés : *Alimentation animale, micro-organismes, probiotiques, croissance, efficacité alimentaire, santé animale.*

Introduction

The use of foods derived from microbial activity goes back to the dawn of human civilization and fermented milks were probably the first foods to contain active microorganisms.

The beneficial effect of fermented milk was given a scientific basis at the beginning of the XXth century by Elie Metchnikoff. Interest in the gut microflora revived after the Second World War and it was shown that supplementing diets with little amounts of antibiotic increased the growth of the animals.

The progressive reduction of the use of antibiotics in animal feed, as growth promoters, has raised renewed interest in the incorporation of microbial strains in animal feed, in order to maintain the beneficial effect obtained with antibiotics.

The term "probiotic" was proposed by Parker (1974) for the first time. The definition of "probiotic" has changed several times over the years, but was recently revised so as to indicate the necessity that the microbial cells be viable.

Probiotics are live microorganisms that, when administered through the digestive route, are favorable to the host's health (Guillot, 1998).

Microorganisms used in animal feed

The microorganisms used in animal feed are mainly bacterial strains of Gram-positive bacteria belonging to the types *Lactobacillus, Enterococcus, Pediococcus* and *Bacillus.* Some other probiotics are microscopic fungi such as strains of yeasts belonging to the *Saccharomyces cerevisiae* species (Fuller, 1992; Guillot, 1998; Thomke and Elwinger, 1998).

It is advisable to notice that among the bacterial species used as probiotics, the *Bacillus* and the *Lactobacillus* differ in many characteristics.

Moreover, *Lactobacillus* and the *Enterococcus* are bacterial families present in great quantities, 10^8 and $10^5/10^6$ per gram respectively, in the digestive microflora of animals. On the other hand, the *Bacillus* and the yeasts are not usual components of the gut microflora (Ducluzeau and Raibaud, 1979; Guillot and Ruckebusch, 1994).

Colonization ability

Because among the probiotics are microorganisms that are usual host of the digestive tract (*Lactobacillus* and *Enterococcus*) and others that are not (*Bacillus, Saccharomyces*), we have studied the ability of some strains of probiotics to colonize the gut of axenic and gnotoxenic chickens (Guillot, 1998).

A probiotic strain of *Enterococcus faecium* is able to colonize the axenic and gnotoxenic gut after a single administration. The population size of the strain in the intestines of gnotobiotic animals is similar to the population size of the resident *Enterococcus.* Similar results were obtained with strains of *Pediococcus* and *Lactobacillus.*

For *Bacillus*, spores administered to axenic and gnotobiotic animals do not colonize the gut and are referred as transients. Continuously administered in feed, the bacterial strain is diluted in the proportion of a decimal logarithm but present in the different parts of the gut.

Dose and mode of administration

A bacterium will have an activity in the gut if the concentration is outstanding, that is to say in great number so that the quantity of substances it produces, such as amino acids, vitamins, antimicrobial molecules, should be sufficient to have an action (Ducluzeau and Raibaud, 1979).

Many microbiologists consider that unless it has 10^6-10^7/g in the intestinal contents, the density is not sufficient to obtain a balance between the probiotic and the bacteria of the resident flora and to have a considerable activity on the host. Although the physiology of the bacteria in the gut is not well known, this close estimate gives an idea of the bacterial population size to be reached to obtain a possible effect.

For many animal species the mode of administration has mainly been feed or drinking water. Feeding by means of spray in the hatcheries is also recommended in breeding conditions so as to obtain one day old chicks contaminated by probiotics. Feed is the safest way to get the right proportion and quantity to be introduced daily.

Continuous feeding over the breeding period is the rule to keep steady and high probiotic rate permanently in the gut. Feeding tests of *Lactobacillus* every second day in drinking water don't show any change in the results obtained. On the other hand the consumption of pelleted feeds by poultry requires the perfecting of technological methods in the order to protect non sporulated bacteria such is the case for *Lactobacillus, Enterococcus* and *Pediococcus* (Tournut, 1989, 1993;

Guillot, 1993).

Mode of action

The gut microflora forms with its host animal a complex ecosystem and microbial interactions ensure the stability of the ecosystem and the health of the host. In some cases the gut microflora is unbalanced and the biological defences against pathogenic agents less effective.

The introduction of a probiotic in the gut is an unnatural event which will act on the natural and complex interactions of the microbial flora.

The global positive effects observed are better zootechnical results: weight gain, feed conversion.

Currently little is known about the way in which probiotics work but, according to the general knowledge of gut microecology, several ways in which probiotic supplements may be influencing the composition of the gut microflora and affecting the health of the host are suggested.

The positive effect observed can be the result of either a direct nutritional effect, similar to the effect obtained with antibiotics, or a "health" or sanitary effect, where the probiotic act as a bioregulator of the gut microflora and reinforces the natural host defences.

The different mechanisms of action suggested are:

(i) *Nutritional effect*

- Reduction of metabolic reactions that produces toxic substances.
- Stimulation of indigenous enzymes.
- Production of vitamins or antimicrobial substances.

(ii) *Sanitary effect*

- Increase in colonization resistance.
- Stimulation of the immune response.

Most of the mechanisms of action are still only hypotheses and need to be accurately demonstrated. Few of them were demonstrated *in vitro* or on laboratory animals (Garvie *et al.*, 1984).

Some experiments have demonstrated *in vitro* the effects of strains of *Saccharomyces cerevisiae* on the activity of anaerobic rumen microorganisms. The addition of *S. cerevisiae* live cells to cultures of some cellulolytic fungal species stimulated zoospores germination and cellulose degradation. The addition of yeasts stimulate also the growth of some anaerobic bacteria, including the cellulolytic and the lactic acid utilising bacteria (Chaucheyras *et al.*, 1995; Yoon and Stern, 1996).

Efficiency of the probiotics

It is known that probiotics can be useful for animal husbandry, but it is also known that the preparations being used do not always exert reproducible effects (Thomke and Elwinger, 1998).

Effectively results of field trials with probiotics are frequently divergent in many animal species, particularly in pigs and cattle (Wolter and Henry, 1987; Jonsson and Conway, 1992).

When there are control groups, quite often the difference observed between them and the treated group is not statistically significant. This is due to the fact that when probiotics have a positive effect on growth, it is reduced and usually inferior to that obtained with antibiotics.

Therefore the efficient measures have to be carried under conditions of controlled breeding so as to look for a nutritional or a sanitary effect.
For example, under similar conditions we have studied the efficiency of a strain of *Bacillus* and

Enterococcus on the growth of chickens. The *Bacillus* has improved growth of 1.5% versus 2.1% for bacitracin, whereas the strain of *Enterococcus* has reduced it of about 1.7% (Guillot and Yvoré, 1990). These breeding conditions, which exclude the phenomena linked to the environment, show the unquestionable but low efficiency of some probiotics.

Several tests carried out on breeding conditions confirm that in poultry, as well as in other animal species, various kinds of stress and subclinical pathology reduce the zootechnical performances of animals and therefore probiotics have a superior activity under these conditions.

In gnotobiotic breeding conditions, we have carried out a controlled caecal coccidiosis with *Eimeria tenella* associated with *Salmonella* carriage and tested the eventual effect linked to the administration to the chickens of a strain of *Bacillus.* We have observed a reduction of the clinical symptoms linked to a better growth in the groups receiving *Bacillus* spores. The results obtained were reproducible and statistically significant. However, this efficiency is not to be compared to the effect of anticoccidial drugs or antibiotics and no differences against *Salmonella* carriage was observed between treated or untreated animals in this experiment (Guillot *et al.*, 1990).

We have also studied some probiotics in rabbit breeding. Rabbit breeding is characterized by the high frequency of digestive disorders which reduce the zootechnical performances.

The probiotics used concern mainly spores of *Bacillus* and *Saccharomyces cerevisiae* incorporated in feed.

The results obtained with a strain of *Bacillus* tested in conventional breeding conditions were:

(i) An increase of growth and a better feed conversion rate.

(ii) a decrease of culled does associated to an inferior feed consumption in the treated group.

The use of a strain of *Saccharomyces cerevisiae* in pelleted feed shows also a positive effect in animal fattening. Besides, this effect is all the more noticeable as the animals are less in protected conditions and more in usually breeding conditions.

This efficiency chiefly means a lower death rate.

Guidelines for the use of probiotics in animals

Since 1970, the use of additives in animal feedstuffs is regulated in Europe (Directive 70/524/EEC). This directive was adapted in 1994 to include the microorganisms and enzymes (Directive 94/40/EC) (Rosen, 1996).

The principal modifications introduced to the guidelines taking out the assessment of microorganismes and enzymes as additives are related to specifications necessary for the identification and characterization of the active substance and to studies to be carried out to guarantee the safety of the use of the product.

Concerning the safety:

(i) Microorganisms must be free of diffusible antibiotic resistance genes and non-pathogenic and non-toxinogenic for target species and for man under expected conditions of use.

(ii) Some studies on target species are required concerning toxicological and microbiological aspects namely tolerance test and tests to determine the effect on the colonization of the digestive tract.

Since 1996, the request for authorization should be accompanied by a dossier drawn up in accordance with the guidelines and at present the opinion of the Scientific Committee for Animal Nutrition (SCAN) on the safety of the microbiological strains is needed by the European Commission for each probiotic.

Conclusion

In spite of the unknown data on the physiology of the gut microflora, we begin to understand with the obtained results in probiotic use more in gut microflora variations. We begin to know how probiotics must be used and which results must be expected.

In the future, a perfect knowledge of the mechanisms of action of each probiotic will make it possible to improve them. Indeed, despite their present low efficiency, it can be a starting point to achieve more efficient microbial strains thanks to todays's biotechnological processes.

References

Chaucheyras, F., Fonty, G., Bertin, G. and Gouet, P. (1995). Effects of live *Saccharomyces cerevisiae* cells on zoospore germination, growth and cellulolytic activity of the rumen anaerobic fungus *Neocallimastix frontalis. Curr. Microbiol.,* 31: 201-205.

Ducluzeau, R. and Raibaud, P. (1979). *Ecologie Microbienne du Tube Digestif.* Masson, Paris.

Fuller, R. (1992). *Probiotics. The Scientific Basis*. Chapman & Hall, London.

Garvie, E.L., Cole, C.B., Fuller, R. and Hewitt, D. (1984). The effect of yoghurt on some components of the gut microflora and the metabolism of lactose in the rat. *J. Appl. Bacteriol.*, 56: 237-245.

Guillot, J.F. (1993). Practical use of probiotics in poultry and rabbits. In: *Microorganisms and enzyme preparations in animal nutrition*, Castanon, J.R. (ed.). EC Report, Brussels, pp. 67-75.

Guillot, J.F. (1998). Les probiotiques en alimentation animale. *Cahiers Agricultures*, 7: 49-54.

Guillot, J.F., Julé, S. and Yvoré, P. (1990). Effect of a strain of *Bacillus* used as a probiotic against *Salmonella* carriage and experimental coccidiosis in chickens. *Microecol. and Therapy*, 20: 19-22.

Guillot, J.F. and Ruckebusch, Y. (1994). Microflore digestive des animaux. In: *Bactéries Lactiques,* de Roissard, H. and Luquet, F.M. (eds). Lorica, Uriage, pp. 343-367.

Guillot, J.F. and Yvoré, P. (1990). Etudes expérimentales des probiotiques chez les volailles. *Bull. des G.T.V.*, 90: 33-37.

Jonsson, E. and Conway, P. (1992). Probiotics for pigs. In: *Probiotics. The Scientific Basis*, Fuller, R. (ed.). Chapman & Hall, London, pp. 259-316.

Parker, R.B. (1974). Probiotics, the other half of the antibiotic story. *Animal Nut. and Health*, 29: 4-8.

Rosen, G.D. (1996). Feed additive nomenclature. *World Poultry Sci. J.*, 52: 53-57.

Thomke, S. and Elwinger, K. (1998). Growth promotants in feeding pigs and poultry. III. Alternatives to antibiotic growth promotants. *Ann. Zootech.*, 47: 245-271.

Tournut, J. (1989). Applications of probiotics to animal husbandry. *Rev. Sci. Tech. Off. Int. Epiz.*, 8: 551-566.

Tournut, J. (1993). Practical use of probiotics in piglets and pigs. In: *Microorganisms and enzyme preparations in animal nutrition*, Castanon, J.R. (ed.). EC Report, Brussels, pp. 77-86.

Wolter, R. and Henry, N. (1987). Bactéries lactiques et alimentation animale. *Bull. Inf. Station Exp. Aviculture Ploufragan*, 27: 108-119.

Yoon, I.K. and Stern, M.D. (1996). Effects of *Saccharomyces cerevisiae* and *Aspergillus orysae* culture on ruminal fermentation in dairy cows. *J. Dairy Sci.*, 79: 411-417.