ALTERNATIVES TO ANTIBIOTIC GROWTH PROMOTERS IN RABBIT FEEDING: A REVIEW.

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ABSTRACT: This review is focused on the most studied and developed substances which are commonly known as alternatives to dietary antibiotics, particularly as far as rabbit feeds are concerned. After a reminder of the reason to be and success of antibiotic growth promoters, and why they lately came to be banned in the European Union, we successively deal with probiotics, prebiotics, enzymes and organic acids. Data on rabbits are, as expected, quite scarce when compared to species such as pigs and poultry. Nevertheless, the available performance results are discussed together with the possible mechanisms of action. Special mention is made of the effects of these substances on digestibility and caecal activity.

Key words: Probiotics, prebiotics, enzymes, organic acids, rabbits

INTRODUCTION

The use of antibiotic growth promoters (AGPs) in animal production began half a century ago, when Stokstad and Jukes added residues of chlortetracycline production to chicken feed. They were added with the objective to serve as a source of vitamin B₁₂, but they caused a growth stimulation that was far too large to be explained only as a vitamin effect (review by Brezoen et al., 1999). The almost obvious cause lay in the antibiotic activity of the residues. This observation was quickly extended to other antibiotics and to other animal species, leading to widespread adoption of AGP inclusion in feeds.

During the last decades considerable amounts of antibiotics were used in animal production, both as therapeutic and as growth promoting agents. Therapeutic usage of antibiotics is typically a high dose-short term one, the substance being either injected, or administered via feed or water. Growth-promoting usage is typically the opposite, i.e., a low dose-long term administration, usually given in feed. A certain degree of overlapping exists of course between the two usages. Prophylactic usages, while intentionally therapeutically, can resemble growth-promoting usages, and the latter can have a degree of prophylactic action. On the other hand, growth promotion in short-lived species (e.g. broilers) is necessarily short-term. In the former EU legislation, the two usages were strictly segregated. AGPs were a small, and lately vanishing, subset of the whole antibiotic arsenal. Still, they more or less consistently improved the production performances, with most of the economic benefits being passed to consumers, via lower prices of meat, eggs, and other animal products. AGPs also had secondary advantages, which are often forgotten. By decreasing feed usage per production unit,
AGPs can reduce the amount of land needed for feedstuff production, the imports of feedstuffs of many countries, and the manure volume (manures are a liability in many modern production systems). Most AGPs used in cattle production can also reduce methane emissions.

Though a lot of research was done on the modes of action of AGPs, less is known about them than about their practical effects. Dozens of mechanisms were proposed over the years, some of them specific to ruminants, and it is likely that most, if not all of them, can contribute to the overall result. The fact that germ-free animals usually do not respond to AGPs strongly suggests that their main and/or immediate actions occur in the gut microbial ecosystem. It is no wonder then that there is still no final agreement on the mechanisms of action of AGPs: they are acting in an extraordinarily complex system, definitely more than thought only few years ago, before the widespread adoption of molecular techniques of microbiological identification. Furthermore, interactions between the microbes and the gut immune system, another very complex and incompletely understood one, can only add to the difficulties of the subject.

Table 1, mostly based on the 1997 Commission on Antimicrobial Feed Additives report (1997), but also on Barton (2000) and Gaskins et al. (2002), summarizes many of the effects of AGPs, e.g., a degree of inhibition of pathogenic microorganisms, a reduction in microbial toxic metabolites, a lowering of the turnover of the epithelium, a nutrient-sparing effect, and a reduction in intestinal motility. Still another often mentioned mechanism is a reduction in the bacterial deconjugation of bile salts.

Two of the former mechanisms are worth stressing: the lower epithelial turnover, because a significant part of the energy and nutrients of the diet is used in the maintenance of the gut; and the inhibition of pathogens, because it is usually assumed that AGPs are used much below the antibiotic minimum inhibitory concentrations. It is commonly assumed that AGPs help to reduce the disease burden, thus improving performances and animal welfare, and a disease-preventing and/or a subclinical disease reducing effect is partly supported by the common observation that AGPs efficiency is inversely related to the hygienic conditions of the farm (Barton, 2000).

At the farm level AGPs cause improvements in feed efficiency, in growth rate, and in egg production. Improvements in growth rate and feed conversion rate (FCR) happen even when intake is maintained constant, which points to a specific effect on efficiency, probably related to nitrogen metabolism.

**Table 1:** Some of the physiological, nutritional and metabolic effects attributed to antimicrobial feed additives (Commission on Antimicrobial Feed Additives, 1997)

| Physiological effects | Nutritional effects | Metabolic effects |
|-----------------------|---------------------|------------------|
| Gut food transit      | R                   | I                |
| Gut wall diameter     | R                   | R                |
| Gut wall length       | R                   | I                |
| Gut wall weight       | R                   | I                |
| Gut absorptive capacity| I                   | I                |
| Faecal moisture       | R                   | I                |
| Mucosal cell turnover | R                   | I                |
| Stress                | R                   | I                |
| Feed intake           | R = I               | I                |

R: a reduction; =: no effect; I: an increase
ALTERNATIVES TO ANTIBIOTIC GROWTH PROMOTERS

(Gaskins et al., 2002). This might help to explain the fact that young animals usually give stronger responses to AGPs than the older ones.

DIETARY ANTIBIOTICS IN RABBITS

It was initially thought that AGPs, being antimicrobial in nature, would be necessarily counterproductive in animal species where a very significant part of digestion is done by microbes. This reasoning mainly applies to ruminants, but can be extended to hindgut-fermentation herbivores, such as the rabbit. Practical experience, supported by scientific evidence, showed that this was not necessarily the case. Some AGPs improve the performances of ruminants and rabbits. Zinc bacitracin was the most used AGP in rabbit feed.

THE ANTIBIOTIC BAN

Although frequently unsound, increasing worries with food safety led European consumers to oppose the usage of AGPs in animal feeds. Part of the worry with the AGPs had to do with eventual antibiotic residues in meat, milk and eggs. As a matter of fact, the antibiotics that were cleared for use as AGPs in the EU had practically no intestinal absorption. Insufficient absorption, together with a limited antimicrobial spectrum, and a lack of critical therapeutic applications (either human or veterinary) were prerequisites for the approval of AGPs in Europe. Problems with antibiotic residues sometimes occur particularly in milk, but in this case related to local udder treatments, not to in-feed AGPs.

Other consumers, together with a significant part of the medical profession, pointed out that AGPs, being antibiotics administered at low doses and during long time intervals, could only lead to microbial resistances in farm animals. This is a significant criticism and one that is supported by scientific evidence (Wegener, 2006). AGPs select for resistant strains and the fact that different species of bacteria can interchange genetic material only makes matters worse. But controversy still exists as far as the practical significance of these resistances is concerned, and in particular about its contribution to the escalating, and worrying problem of antibiotic resistance in pathogenic organisms. Its contribution is possibly minor, especially when compared to massive, but by and large unavoidable, antibiotic usage in hospitals, and to inadequate and partly avoidable antibiotic usage by consumers, general practitioners, and field veterinarians. Yet, the environment was ripe for AGP interdiction.

Scandinavian countries, starting with Sweden in 1986, were the first to ban AGPs. Their partial success in curbing resistances surely contributed to the general EU ban of AGPs, starting in 2006, and the fact that such a ban is now a considered hypothesis in the USA. It cannot and should not be forgotten, however, that the Scandinavian ban was only a part of a series of weapons of a global strategy against bacterial resistance to antibiotics, which also included serious efforts geared towards the medical profession, and the general public.

Scandinavia was a test bed for the ban of AGPs. After an initial period, when the withdrawal of AGPs was partly compensated by increasing therapeutic usage of antibiotics in farms, farmers and technicians adapted to a new reality, finally reducing, as intended, global antibiotic usage. A number of feed additives, commonly described as antibiotic alternatives, helped to ease the transition to this new reality.

At the same time, growing criticism of AGP utilization in animal production fuelled the search for non-antibiotic substances, which might have similar effects in food-producing animals. Because this was the main stimulus for their study and development, they are commonly referred to as alternatives to antibiotics, yet there is little in common between them, and they are often interesting in their own, with or without antibiotic ban. Among the many alternatives, probiotics, prebiotics, symbiotics,
enzymes and organic acids were perhaps the most studied and developed. The first three, which may be considered as a group, have also been much looked upon from a human nutrition and health point of view. But other alternative products can be mentioned, such as immune system stimulators and plant or herbal extracts.

Interest in these alternative products grew significantly in the eighties. Understandably, performance studies initially outnumbered mode of action studies, but a significant amount of research has already been done on the latter often in vitro or with laboratory animal tests, less frequently with farm species. After initial, and then often justified, distrust of these products by animal nutritionists and veterinarians, they became generally and rightly accepted, to the point that the EU feed additive legislation was altered to make room for them.

In common with antibiotics, most if not all of these alternative products can act upon the gut microbiota, and the gut immune system; and, thus being, their mode of action will probably turn out to be as complex to unravel as the mode of action of AGPs. Another possible common point is the fact that they can be more useful and cost-effective in certain critical periods (e.g. weaning) and/or when environmental conditions are suboptimal.

In the monogastric camp, alternatives to antibiotics were, as expected, mostly studied in pigs and poultry (Thomke and Elwinger, 1998; Doyle, 2001). Because of the peculiar digestive physiology of the rabbit, it can be hazardous to simply extend the conclusions of such studies to this species. We shall stress some differences when they are relevant.

**PROBIOTICS**

Interest in probiotics is invariably traced back to Elie Metchnikoff’s studies about the potential benefits of fermented milks in human nutrition, in the beginning of the twentieth century. The word probiotic itself was introduced much later. There has been some controversy regarding the operational definition of probiotics (e.g. kind of microorganisms, whether they have to be alive or not), but widely accepted is the definition as a preparation of live microorganisms which, when administered in adequate amount, have beneficial effects on the health of the person or animal (Hamilton et al., 2003).

Several reviews (e.g. Ziemer and Gibson, 1998; Ouwehand et al., 1999; Simon et al., 2003) have suggested a number of possible mechanisms of action of probiotics, among which a reduction of metabolic reactions which produce toxic substances, the stimulation of host enzymes, the production of vitamins or antimicrobial substances, the competition for adhesion to epithelial cells and an increased resistance to colonization, and the stimulation of the immune system of the host.

A number of studies mention the production of a range of antimicrobial substances by probiotic bacteria. Among such substances are organic acids, hydrogen peroxide and bacteriocins, which can kill other microbes, alter their metabolism, and/or reduce their production of toxins (Rolfe, 2000). But it should not be forgotten that some of these mechanisms were verified in vitro, and so need to be substantiated in vivo to be more than hypothesis (Thomke and Elwinger, 1998; Guillot, 2001).

In vivo studies with farm species have mainly looked at performances and health status, but some work has been done on the effects of probiotics on the gut microbiota, including pathogenic species, and on the gut morphology and physiology. In some studies, animals were used as a human model (Thomke and Elwinger, 1998).

Most microorganisms used in probiotics are strains of Gram-positive bacteria of the genera *Bacillus* (*B. cereus*, var. *toyoi*, *B. licheniformis*, *B. subtilis*) *Enterococcus* (*E. faecium*), *Lactobacillus* (*L.*
Alternatives to antibiotic growth promoters

acidophilus, L. casei, L. farciminis, L. plantarum, L. rhamnosus), Pedicoccus (P. acidilactici) and Streptococcus (S. infantarius). Some yeast and fungi are also used, most frequently some strain of Saccharomyces cerevisae.

A significant number of trials show positive effects of probiotics, especially among younger animals, such as chicks and piglets, raised in less than ideal hygienic conditions (reviewed by Thomke and Elwinger, 1998, and Simon et al., 2003). But a certain number without positive or even negative effects have also been published (Doyle 2001).

The lack of consistency in results can be traced to an important number of causes, some of them related to the animal, others to the probiotic. Among the former, and apart from natural individual differences (Simon et al., 2003), one may list all the factors that may influence the animal gut microbiota, such as diet, stress and/or disease. Among the latter, the choice of species and strains, the technological preparation of the probiotic, the manufacturing of the feed, the dose of administration, and interactions between probiotic and drugs, just to name a few.

The live bacteria and/or yeasts of the probiotic must be able to withstand the manufacture and storage of the feeds where they are included. This is particularly critical with non-spore forming bacteria (e.g. Lactobacillus, Pedicoccus and Streptococcus). At least one commercial product sold for ruminants is based on recognisably dead microorganisms, and as such is free from these prerequisites. Whether it should be technically be considered a probiotic is open to question.

Also, probiotics must resist the animal digestive secretions and present no risk of toxicity for it. According to Guillot (2001), probiotic organisms must attain concentrations in the order of 10^6-10^7 per g in the intestinal content to have any observable effect.

The formulation of the feed can be tailored so as to maximize the effect of a probiotic, and this is in a certain way the basis for symbiotics % preparations containing a combination of a probiotic and a prebiotic. Working with piglets, Bomba et al. (2002) showed that maltodextrins and polyunsaturated fatty acids could potentiate probiotics in the small intestine, while fructo-oligosaccharides (FOS) could potentiate them in the large intestine.

**Probiotics for rabbits**

There are naturally fewer studies with probiotics in rabbits than in other monogastric farm species. Several studies exist nevertheless, which are limited to the assessment of the effect on growth, feed conversion, reproduction and mortality; sometimes caecal activity and digestibility are studied too.

Table 2 synthetically describes a reasonable number of experimental trials with probiotics in growing and fattening rabbits. The results of these trials were used to make the graphs depicted in Figure 1, which show average daily gain (ADG), feed conversion ratio (FCR), and mortality, respectively. ADG and FCR are expressed as a percentage of the controls; mortality as the absolute difference, in percentage points, between a treatment and the corresponding control.

It is worthwhile noting that, while it is true that the differences were not statistically significant in many trials, improvements in ADG were obtained in 15 out of 20 trials. It can be added that in one of the only two negative result trials, the diet was deficient in fibre (only 10% of ADF). The same can, by and large, be said about FCR. Mortality was also reduced in a great part of the trials where it was measured (7 positive, 6 null, and 3 negative results).

The number of trials where reproduction results were studied is smaller. The results are summarized in Table 3 and suggest that the main effect can be an increase in litter weight at weaning; but the differences were not always statistically significant.
Table 2: A summary of some experimental protocols used in probiotic trials with growing and fattening rabbits

| Reference                | Days on trial | Probiotic                | No. trial | Probiotic level | Unit Rabbits/cages | Dietary fibre | Trial conditions                        |
|--------------------------|---------------|--------------------------|-----------|-----------------|-------------------|--------------|-----------------------------------------|
| Luick et al., 1992       | 36 d          | Lacto-sacc (2)           | 1         | 0.2%            | 15                | 23.1% ADF    | Experimental                            |
| Luick et al., 1992       | 36 d          | Lacto-sacc(2)            | 2         | 0.2%            | 14                | 9.9% ADF     | Experimental/low fibre                   |
| Gippert et al., 1992     | 28-84 d       | Lacto-sacc (2)           | 3         | 0.1%            | 172               | 10.6% CF     | Commercial                              |
| Gippert et al., 1992     | 42-77 d       | Lacto-sacc(2)            | 4         | 0.1%            | 100               | 10.6% CF     | Experimental                            |
| Yamani et al., 1992      | 28-84 d       | Lacto-sacc(2)            | 5         | 0.1%            | 24                | 16.7% CF     | Commercial                              |
| De Blas et al., 1991     | 30 d-2 kg LW  | Paciflor *(Bacillus CIP 5832)* | 6         | 0.01% (10^6 spores/g) | 45                | 36.5% NDF    | Between 23-28°C and 18-22°C             |
| Maertens and De Groote, 1992 | 28-70 d       | Biosaf *S. cerevisae*    | 7         | 0.15%           | 60                | 15.5% CF     | Optimal housing conditions               |
| Maertens et al., 1994    | 28-70 d       | Paciflor *(Bacillus CIP 5832)* | 8         | 1%              | 60                |              |                                         |
| Maertens et al., 1994    | 28-70 d       | Paciflor *(Bacillus CIP 5832)* | 9         | 0.15%           | 93                | 15.5% CF     |                             |
| Maertens et al., 1994    | 28-70 d       | Paciflor *(Bacillus CIP 5832)* | 10        | 1%              | 95                |              |                                         |
| Jerome et al., 1996      | 30-79 d       | *Saccharomyces cerevisae* | 13        | 10^6 spores/g   | 18 cages with 6 rabbits | 16.5% CF     | Experimental                            |
| Kustos et al., 2004      | 35-77 d       | Bioplus 2B *(B. licheniformis, B. subtilis)* | 14        | 0.04% (1.28 x 10^6 cfu/g) | 60                | 15.5% CF     | Experimental 18-23°C - Thermal stress   |
| Amber et al., 2004       | 35-126 d      | Lact-A-Bac *(L. acidophilus)* | 15        | 0.05% (8 x 10^11 cfu/g) | 27                | 12.5% CF     | Experimental                            |
| Trocino et al., 2005     | 35-70 d       | Toyocerin *(B. cereus var. toyoi)* | 16        | 0.02% (2 x 10^8 spores/g) | 63 cages | 41% NDF | Commercial |
| Esteve-García et al., 2005 | 28 d          | Toyocerin *(B. cereus var. toyoi)* | 17        | 0.1% (1 x 10^9 spores/g) | 62 cages |              |                                         |
|                          |               |                          | 18        | 0.02%           | 15 x 5 cages     | 14.9% CF     | Experimental                            |
|                          |               |                          | 19        | 0.05%           | 15 x 5 cages     |              |                                         |
|                          |               |                          | 20        | 0.10%           | 15 x 5 cages     |              |                                         |

1) Number of trial in the graphic 1, 2, and 3. 2)Lactobacillus acidophilus + Streptococcus faecium + yeasts + enzymes (protease, cellulase, amilase) LW= live weight. cfu= colony-forming units.
The effect of probiotics on digestibility was addressed by several researchers. While neither Gippert et al. (1992) nor Luick et al. (1992) found any effect, others did. In the trial of Yamani et al. (1992), Lacto-Sacc (a complex product containing microorganisms % Lactobacillus acidophilus, Streptococcus faecium and yeasts % but also enzyme activities % protease, cellulases, amylase) improved crude fibre digestibility at 8 and 12 weeks. Amber et al. (2004), working with Lact-A-Bac (Lactobacillus acidophilus), got improvements in the digestibilities of energy and of most analytical fractions (DM, CP, EE), including crude fibre.

Since probiotics can influence gut microbiology, several authors looked at their effects upon the caecum microbiota, either by counting bacteria (Amber et al., 2004) or their products, VFA in particular (Maertens et al., 1994). In the study of Amber et al. (2004), the probiotic significantly increased cellulytic bacteria counts (cfu/ml), while at the same time decreasing the counts of ureolytic ones. In this study, caecal pH was unaffected by the probiotic. In the study of Maertens et al. (1994), the probiotic Paciflor did not affect either pH or VFA caecal levels.

To better understand the effects, and to design better probiotics for rabbits, it will be necessary to apply to this species the kind of studies which are already common in humans, in laboratory animals, and in other farm species. Research must be done on details referred by Klis and Jansman (2002) such as lumen physico-chemical conditions and enzyme activities; morphology, absorption capacity and barrier effect of the epithelium and also in the effect in status of the gut immune system. The same reasoning applies to prebiotics and symbiotics, discussed in the next section.
At this moment there are only two probiotics approved for rabbits in the EU. One of them is bacterial, i.e. *Bacillus cereus* var. *toyoi*, the other is a yeast, i.e. *Saccharomyces cerevisiae* NCYC Sc 47.

**PREBIOTICS**

Prebiotics are another possible alternative to antibiotics. A very recent term, prebiotic usually refers to oligosaccharides which are not digested by the animal enzymes, but can selectively stimulate certain intestinal bacteria species, which have potential beneficial effects on the host health. Prebiotics can be either directly extracted from natural sources (plants, yeasts, milk), or be produced by partial acid or enzymatic hydrolysis of polysaccharides or by transglycosylation reactions (Oku, 1996). The main commercial oligosaccharides are nowadays the fructo-oligosaccharides (FOS), the α-galacto-oligosaccharides (GOS), the transgalacto-oligosaccharides (TOS), the mannan-oligosaccharides (MOS) and the xilo-oligosaccharides (XOS)

While probiotics are meant to bring beneficial microbes to the gut, oligosaccharides are supposed to selectively stimulate the beneficial microbes that already live there. They have two clear advantages relative to probiotics: a technological one, because there are no critical problems with the thermal processing of the feed and the acid conditions of the stomach, and a safety one, because they do not introduce foreign microbial species into the gut. Beneficial microbes, if stimulated, will better be able to compete with the undesirable ones. But prebiotics can also have other beneficial effects, irrespective of stimulating that part of the gut microbiota (Forchielli and Walker, 2005): firstly, they can prevent the adhesion of pathogens to the mucosa, by competing with its sugar receptors, and secondly they can directly stimulate the gut immune system.

The mode of action of prebiotics has been mainly studied *in vitro* and with laboratory animals, and most of the works published relate to human foods. Positive effects have been found in farm animals, such as improvements in daily gain, feed conversion ratio and/or health status, but the effect tends to vary with the oligosaccharide and the conditions of utilization (Patterson and Burkholder, 2003; Lan *et al.*, 2005).

**Prebiotics for rabbits**

Some prebiotics were already tested in rabbits. Most of the works published to date concern their effects on the production performances, and/or the caecal microbiota; more recently, there has been work on their effects on gut morphology.

The effects of prebiotics on rabbit performances have been at best inconsistent. As far as FOS are concerned (Table 4), Aguilar *et al.* (1996) got a positive effect on growth rate, without effect on FCR; Mourão *et al.* (2004) found the opposite, i.e., no effect on growth rate but a tendency for an improvement in FCR; differently, Lebas (1996) did not get any effect at all. Results were also null for Peeters *et al.* (1992), working with GOS, whereas Gidenne (1995) got a significant negative effect of GOS on morbidity and mortality.

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**Table 3:** Effect of probiotics on reproductive performances (differences in % of control group) (adapted from Maertens *et al.*, 2006).

| Reference                        | Probiotic                        | Parturition interval | Litter weight at weaning | Litter size at weaning |
|----------------------------------|----------------------------------|----------------------|--------------------------|------------------------|
| Maertens and De Groote, 1992     | Biosaf                           | +3.5                 | +1.3                     |
| Maertens *et al.*, 1994          | Paciflor *Bacillus* cip 5832     | +6.4*                | -1.3                     |
| Nicodemus *et al.*, 2004         | *Bacillus cereus* var. *toyoi*   | -10.2*               | +7.6                     | +9.9                   |
| Pinheiro *et al.*, 2006          | *Bacillus cereus* var. *toyoi*   | +5.4                 | -3.3                     |

*P<0.05
In rabbits, prebiotics should create unfavourable conditions for pathogenic microorganisms in the caecum. A few research trials were conceived with this objective in mind. The results of Morisse et al. (1992) are supportive of a barrier effect of FOS in the caecum: the saprophyte *E. coli* population increased, the VFA production increased, the ammonia levels in caecal contents decreased. But Maertens et al. (2004), testing FOS and inulin, only got an effect on the molar proportions of VFA. As far as other prebiotics are concerned, both GOS (Peeters et al., 1992) and MOS (Mourão et al., 2006) were able to increase the caecal VFA levels. On the contrary Gidenne (1995) did not observe any effect of GOS addition on cecal VFA pattern.

Fructans with a lower degree of polymerization may be hydrolyzed by microbes residing in the upper intestine, especially when the rabbit is actively practicing caecotrophy (Carabaño et al., 2001). If not, as can happen in very young animals, they shall primarily act in the caecum. Maertens et al. (2004) showed that, when rabbits are not allowed to practice caecotrophy, the ileal digestibilities of FOS and inulin are similar and not far from 50%.

MOS, which are thought to act mainly by preventing colonization more than by stimulating beneficial microorganisms, are considered promising prebiotics (Kocher, 2006). Many pathogens have fimbriae which specifically attach to the mannose residues of intestinal cell receptors and by connecting to MOS instead will not attach to the mucosa. In several trials with MOS (Fonseca et al., 2004; Pinheiro et al., 2004; Mourão et al., 2006), performances were comparable to the ones obtained with AGPs. As to the effect on gut morphology, Mourão et al. (2006) reported that MOS increased the length of ileal villi, possibly a result of the reduction in microbial counts, which they also detected.

Lack of consistency in the results obtained with prebiotics can be explained by differences in the experimental protocols, e.g. number of animals, hygienic conditions, nature of prebiotic, amount of prebiotic added to feed. This latter factor has been stressed by several researchers (e.g. Mourão et al., 2006). If the amounts that must be added are very high, the use of prebiotics is compromised by cost. Also, it is just possible that prebiotics which show benefits in long-living animals, humans in particular, do not show them in short-living species, such as the rabbit.

Last but not least, it should not be forgotten that rabbit diets are naturally rich in fibrous feedstuffs, some of them having significant amounts of oligosaccharides. A possible alternative to commercial prebiotics would be to select the feedstuffs containing the most desirable oligosaccharides for each phase in the rabbit life.

**ENZYMES**

The idea of adding enzymes to feeds is old, but its practical implementation dates from the end of the eighties (Chocț, 2006). Before this period enzymes were not only too expensive, they were tailored for other uses, laundry and food in particular, and as such were generally inadequate, and by consequence useless for the feed industry. Furthermore, most of them were not thermostable and so could not remain active after pelleting of the feed. Add to this the fact that many would not resist the

| Reference          | Average daily gain | Feed conversion | Mortality (%) control -% experim. |
|--------------------|--------------------|-----------------|-----------------------------------|
| Aguilar et al., 1996 | $P < 0.001$ (32.3 vs 35.9 g/d) | NS (3.16 vs 3.10) | NS (6.3 vs 5.9) |
| Lebas, 1996        | NS (35.5 vs 35.6 g/d) | NS (3.30 vs 3.30) | NS (3.3 vs 3.3) |
| Mourão et al., 2004 | NS (40.1 vs 40.6 g/d) | $P < 0.01$ (3.6 vs 3.3) | NS (19.4 vs 16.7) |

1 Very low mortality (1%), not related to the FOS content.
acid of the stomach, and/or the digestive proteases, and it is not hard to understand why they tended to fail in the beginning.

The first successful enzymes to be added to feeds were beta-glucanases and xylanases, which are able to partially hydrolyze non-starch polysaccharides (NSP) of wheat, rye, triticale, oats and barley. The benefits of these enzymes are firmly established in poultry feeding, and their commercial use is already widespread. They reduce the intestinal viscosity caused by beta-glucans and arabinoxylans, respectively, and this in turn improves the absorption of nutrients, the quality of the litter, and the cleanliness of the eggs. Also, they can reduce the available substrates for microbial proliferation in the ileum and caecum, while stimulating the more beneficial organisms, as a result of the oligosaccharides and/or sugars they are able to release (Bedford, 2000). Moreover, these enzymes allow for greater flexibility and thus lower costs in feed formulation, especially when maize is scarce and/or very expensive compared to other cereals. While complete hydrolysis can be desirable in the case of glucans, because it leads to glucose, partial hydrolysis of arabinoxylans can probably suffice, as long as viscosity is satisfactorily reduced.

The second successful enzymes, although on a smaller scale, were the phytases. Phytases not only liberate an otherwise unavailable part of the feed phosphorus, thus reducing the need for phosphates and the excretion of this mineral element, they can also improve the availability of other nutrients. Phytases can be economically interesting when phosphates are scarce and expensive and/or phosphorus levels in manures are taxed.

Other enzyme activities have already been studied, examples being α-1,6 galactosidases and β-1,4-mananases, used to hydrolyze flatus-causing oligosaccharides of soybeans and other legume grains. In a recent review of 14 trials of soybean-based diets for pigs, supplemented with α-1,6 galactosidases, β-1,4-mananases, or enzyme complexes, Kim and Baker (2003) concluded that the enzymes had positive effects on growth performances and digestibility in 70% of the cases.

Glucose-liberating cellulases are perhaps the Holy Grail of feed enzymes, but up till now they have only met partial successful. The large complexity and interlinkages of the cell wall structure of plants are partly responsible for the weak response. Cellulases have been often tested, and are sometimes used, in silages, which also are animal feeds. But enzymes for silages are a special topic, which shall not be discussed here.

Enzymes used in feeds are thus all hydrolases. They should be tailored to the composition of the feed, be heat-tolerant, so as to resist pelleting, be acid-resistant, so as to resist gastric transit, and be resistant to the own proteases of the animal. When interpreting effects, it should not be forgotten that most commercial enzymes are in reality crude extracts which contain a whole series of enzyme activities besides the main and declared one.

Enzymes for rabbits
Most of the trials that were performed during the last decade (Remóis et al., 1996; Fernandez et al., 1996; Pinheiro and Almeida, 2000; Falcão-e-Cunha et al., 2004; Garcia et al., 2005) could not detect any significant effect of enzymes on rabbits performances. The only exception was the decrease in mortality which García et al. (2005) found with proteases and proteases + xylanases (probably reducing protein flow to the caecum). Some positive results were also obtained by other researchers: Eiben et al. (2004), testing cellulases, got improvements in FCR and mortality of rabbits weaned at 23 days of age, whereas ADG was unaffected.

It is interesting to note that in some trials enzymes improved fibre digestibility. Such was the case in the studies of Fernandez et al. (1996) and Bolis et al. (1996). The latter authors got significant
improvements when cellulase and enzyme pool (xylanase, β-glucanase, β-glucosidase, pentosanase, myloglucosidase, acid and neutral protease) was added on NDF (+5%) and ADF (+13%) digestibilities, yet at the same time getting reductions of digestible and metabolizable energies, and nitrogen balance, in comparison with the control diets.

The effects of enzymes on the different parts of the rabbit gut were addressed by several researchers. Sequeira et al. (2000) could only detect a lowering of gastric pH but the enzyme complex composed of amylase, xylanase, β-glucanase and pectinase did not have any effect on the digestive parameters measured. Exogenous enzymes frequently fail to significantly affect enzyme activities in the gastric, intestinal and caecal contents (Sequeira et al., 2000), even in the period succeeding an early weaning (Falcão-e-Cunha et al., 2004).

Although rabbits are better able to digest phytic phosphorus than poultry and swine, they are not the equal of ruminants in this regard. In a trial of Gutiérrez et al. (2000), exogenous phytases improved not only the utilization of phosphorus (+24%), but also increased nitrogen digestibility (+7%). According to these authors, phytases can be useful in rabbit diets.

It is not unlikely that, due to its peculiar digestive physiology, and in particular the fact that caecotrophy casts microbial enzymes along the whole length of the gut (Marouneck et al., 1995), rabbits be less responsive than other animals to supplementation with exogenous enzymes. This does not entirely rule out the interest of supplementation, but probably restricts it to particular phases in the life of the rabbits.

ORGANIC ACIDS

Organic acids and salts have a long-history in the food and the feed industries, which commonly use them as preservatives. Some authors also consider them to be a viable alternative to antibiotics, in pig feeds namely, where they already have had considerable success. According to Partanen and Mroz (1999), formic, acetic, propionic, butyric, lactic, sorbic, fumaric, tartaric and citric are the most promising of organic acids in this regard.

The so called acidification started with piglet diets and was thought as a means to compensate the relatively low gastric production of acid of the young animals, particularly when subject to early weaning. It was later verified that it could also been advantageous in the later phases of growth, when they could both improve the apparent digestibility of energy and protein, and the absorption and retention of some minerals (Partanen and Mroz, 1999; Diebold and Eidelsburger, 2006). Several hypotheses have been proposed to explain the positive effects of acidification. The classical ones see the acid as replacing gastric HCl: the activation of proteolytic enzymes, the denaturation and unfolding of feed proteins, the barrier effect against microorganisms brought with the feed. But other non-alternative hypothesis can be mentioned: a residual antimicrobial effect in the lower gut, a specific trophic effect on the intestinal mucosa, an action as nutrients.

The antimicrobial activity of organic acids is basically the same, irrespective of acting in food, feed, or gut lumen (Diebold and Eidelsburger, 2006). Indissociated organic acids easily cross the cellular membrane and tend to dissociate when inside the neutral pH of the microbial cytoplasm. The protons thus liberated can upset the microbial metabolism, namely by inhibition of enzymes and/or transport systems. The efficiency of a given acid depends on its pKa, the pH value at which it will be half dissociated. Higher pKa acids tend to be more effective. On the other hand, the antimicrobial effectiveness of organic acids tends to increase both with their chain length and their degree of unsaturation (as reviewed by Partanen and Mroz, 1999).
The minimum inhibitory concentrations of organic acids for pathogenic microorganisms has been measured \textit{in vitro} (Strauss and Hayler, 2001 cited by Diebold and Eidelsburger, 2006; Mroz, 2005). The results show that the degree of inhibition depends both on the acid and the bacterial species tested.

Responses to organic acids are, however, variable. Part of the differences may have to see with the intrinsic acid activity and buffering capacity of the diets.

Some researchers have also tested medium-chain fatty acids, which also have antimicrobial activity (Decuypere and Dierick, 2003). They were tested either free, or esterified in triglycerides. When esterified, they can be liberated by endogenous or exogenous lipases. This will possibly happen in the intestine, and in this case the gastric mechanisms might be ruled out.

**Organic acid for rabbits**

Studies of organic acids are few, and their results far from consistent, in the case of rabbits (Maertens et al., 2006). Recently, Brazilian researchers (Scapinello et al., 2001; Michelan et al., 2002) found that the inclusion of 1.5% of fumaric acid in the feeds of growing rabbits tended to improve both the daily gain and the feed efficiency, but the differences were not statistically significant. Similar results were reported by Hollister et al. (1990) (Table 5).

A group of Czech researchers have been studied intensively the effects of medium-chain fatty acids. In a study of Skoivannová and Marounek (2002), the inclusion of 0.5% of caprylic acid reduced post-weaning mortality, without affecting any other performance trait. In a later trial, Skoivannová and Marounek (2006), testing the medium-chain fatty acids esterified in triglycerides, reached the same results, i.e. a significant reduction in post-weaning mortality, no effect on feed intake, daily gain, or carcass yield.

Combining organic acids with prebiotics (Scapinello et al., 2001) or with probiotics (Michelan et al., 2002) did not significantly improve performances, though mortality was significantly reduced in one trial (Hollister et al., 1990) where fumaric acid was combined with Lacto-Sacc.

**CONCLUSION**

The amount of research on alternatives to AGPs is limited in rabbits, compared to other farm species. Probably, many studies remain unpublished because of confidentiality, either because of favourable (protection for use with license …) or unfavourable results. Most of the published works have dealt with growth performances, much less with reproduction and mechanisms of action. Although results have often been inconsistent, a number of studies suggest that it will be possible to develop alternatives for this species as well. Because of the complexity of its digestive system, part of the work to be done shall necessarily be by trial and error, but advances in the fundamental modes of action has to lead to species designed alternatives. Also, combinations of two or more of these types of products, as in symbiotics, are still an opportunity to fully explore.
REFERENCES

Aguilar J.C., Roca T., Sanz E. 1996. Fructo-oligo-saccharides in rabbit diet. Study of efficiency in suckling and fattening periods. In Proc.: 6th World Rabbit Congress, Toulouse, France, 73-77.

Amber K.H., Yakou H.M., Hamed Rawya S. 2004. Effect of feeding diets containing yucca extract or probiotic on growth, digestibility, nitrogen balance and caecal microbial activity of growing new zealand white rabbits. In Proc.: 8th World Rabbit Congress, Puebla, México, 737-741.

Barton M.D. 2000. Antibiotic use in animal feed and its impact on human health. Nutr. Res. Rev., 13, 279-299.

Bedford M.R. 2000. Exogenous enzymes in monogastric nutrition – their current value and future benefits. Anim. Feed Sci. Techn., 86, 1-13.

Bolis S., Castrovilli C., Rigoni M., Tedesco D., Luzi F. 1996. Effect of enzyme supplementation in diet on protein and energy utilization in rabbit. In Proc.: 6th World Rabbit Congress, Toulouse, France, 111-115.

Bombá A., Nemcová R., Gancareikova S., Herich R., Guba P., Mudronová D. 2002. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. British. J. Nutr., 88, s95-s99.

Brezoan A., Van Haren W., Hanekamp J.C. 1999. Emergence of a syndrome of interleukin growth of rabbit ileal apparent digestibility of non-starch polysaccharides in food.

Choct M. 2006. Enzymes for the feed industry: past, present and future. World's Poultry Sci. J., 62, 3, 5-15.

Commission on Antimicrobial Feed Additives. 1997. Antimicrobial Feed Additives. Government Official Reports 1997: 132, Ministry of Agriculture Stockholm.

De Blas C., García J., Alday S. 1991. Effects of dietary inclusion of a probiotic (PACIFLOR*) on performance of growing rabbits. J. Appl. Rabbit Res., 14, 148-150.

Decuypere J.A., Dierick N.A. 2003. The combined use of triacylglycerols containing medium-chain fatty acids and exogenous lipolytic enzymes as an alternative to in-feed antibiotics in piglets: concept, possibilities and limitation. An overview. Nutr. Res. Rev., 16, 193-209.

Diebold G., Eidelburger U. 2006. Acidification of diets as an alternative to antibiotic growth promoters. In D. Barug, J. de Jong, A.K. Kies, M.W.A. Verstegen (Eds.) Antimicrobial Growth Promoters. Wageningen Academic Publishers, The Netherlands, 311-327.

Doyle M.E. 2001. Alternatives to antibiotic use for growth promotion in animal husbandry. FRI Briefings, April 2001, 17.

Eiben C.S., Mézes M., Zijátor N., Kustos K., Gödor-Surmann K., Erdélyi M. 2004. Dose-dependent effect of cellulosic supplementation on performance of early-weaned rabbit. In Proc.: 8th World Rabbit Congress, Puebla, México, 799-804.

Esteve-Garcia E., Rafel O., Jiménez G. 2005. Efficacy of Toyocerin® in conechos de engorde. In Proc.: XXX Symposium de Cunicultura de Asesu, Villadolid, 85-89.

Falcão-e-Cunha L., Reis J., Freire J.B., Castro-Solla L. 2004. Effects of enzyme addition and source of fiber on growth and fibrolytic activities of growing-finishi rabbits. In Proc.: 8th World Rabbit Congress, Puebla, México, 1532-1537.

Fernández C., Merino J.M., Carabaño R. 1996. Effect of enzyme complex supplementation on diet digestibility and growth performance in growing rabbits. In Proc.: 6th World Rabbit Congress, Toulouse, France, 163-166.

García A.I., García J., Corrent E., Chamorro, S., Garcia-Rebollar P., De Blas C., Carabaño R. 2005. Effet de l’âge du lapin, de la source de protéine et de l’utilisation d’enzymes sur les digestibilités apparentes de la matière sèche et de la protéine brute sur un aliment lapin. In Proc.: 11èmes Journées de la Recherche Cunicole, Paris, France, 197-200.

Gaskins H.R., Collier C.T., Anderson D.B. 2002. Antibiotics as growth promotants: mode of action. Anim. Biotechnol., 13, 29-42.

Gidenne T. 1995. Effect of fibre level reduction and gluco-oligosaccharide addition on the growth performance and caecal fermentation in the growing rabbit. Anim. Feed Sci. Techn., 56, 253-263.

Gippert T., Virag, G., Nagy, I. 1992. Lacto-Sacc in rabbit nutrition. J. Appl. Rabbit Res., 15, 1101-1104.

Guillot J.F. 2001. Consequences of probiotics release in the intestine of animal. In J. Brufau (Ed.) Feed Manufacturing in the Mediterranean Region Improving Safety: from feed to food. Zaragoza CIEHAM-IAMZ, 17-21.

Gutiérrez I., Espinosa A., García J., Carabaño R., De Blas J.C. 2002. Effects of exogenous phytase on phosphorous and nitrogen digestibility in growing-finishing rabbits. In Proc.: 7th World Rabbit Congress, Valencia, Spain, 277-281.

Hamilton-Miller J.M.T., Gibson G.R., Bruck W. 2003. Some insights into the derivation and early uses of the word “probiotic”. Brit. J. Nutr., 90, 845.

Hollister A.G., Cheeke P.R., Robinson K.L., Patton N.M. 1990. Effects of dietary probiotics and acidifiers on performance of weanling rabbits. J. Appl. Rabbit Res., 13, 6-9.

Jerome N., Mousset J.L., Lebas F., Robart P. 1995. Effect of diet supplementation with oxytetracycline combined or not with different feed-additives on fattening performance in the rabbit. In Proc.: 6th World Rabbit Congress. Toulouse, France, 205-209.

Kim S.W., Baker D.H. 2003. Use of enzyme supplements in pigs diets based on soyabean meal. Pig News and Information, 24, 91-96.

Klis J.D. van der, Jansman A.J.M. 2002. Optimising nutrient digestion, absorption and gut barrier function in monogastrics: reality or illusion. In M.C. Blok, H.A. Vahl, L. de Lange, A.E. van de Braak, G. Hemke, M. HESSING (Eds.) Nutrition and Health of the Gastrointestinal Tract. Wageningen Academia Publishers, The Netherlands, 15-36.

Kocher A. 2006. Interfacing gut health and nutrition: the use of dietary pre- and probiotics to maximise growth performance in pigs and poultry. In D. Barug, J. de Jong, A.K. Kies, M.W.A. Verstegen (Eds.) Antimicrobial Growth Promoters. Wageningen Academic Publishers, The Netherlands, 289-310.

Kustos K., Kovács D., Gödor-Surmann K., Eiben, C.S. 2004. Effect of probiotic Bioplus 2b® on performance of growing rabbit. In Proc.: 8th World Rabbit Congress, Puebla, Mexico, 874-879.

Lan Y., Verstegen S., Tamminga S., Williams B.A. 2005. The role of dietary mannan oligosaccharide in comparison to oxytetracyclin on performance of growing rabbits. In Proc.: 8th World Rabbit Congress, Puebla, México, 829-833.

Forchielli M.L., Walker W.A. 2005. The role of gut-associated lymphoid tissues and mucosal defence. Brit. J. Nutr., 93, s41-s48.

Lebas F. 1996. Effects of fructo-oligo-saccharides origin on rabbit’s
growth performance in 2 seasons. In Proc.: 6th World Rabbit Congress, Toulouse, France, 211-215.

Luick B.R., El-Sayaad A.E., Checke P.R. 1992. Effect of fructooligosaccharides and yeast culture on growth performance of rabbits. J. Appl. Rabbit Res., 15, 1121-1128.

Maertens L., De Groote G. 1992. Effect of dietary supplementation of live yeast on the zootechnical performances of does and weanling rabbits. J. Appl. Rabbit Res., 15, 1079-1086.

Maertens L., Van Renterghem R., De Groote G. 1994. Effects of dietary inclusion of Pacifilr® (Bacillus CIP 5832) on the milk composition and performances of does and on caecal and growth parameters of their weanlings. World Rabbit Sci., 2, 67-73.

Maertens L., Aerts J., De Boever J. 2004. Degradation of dietary oligofructose and inulin in the gastro intestinal tract and the effects on pH and volatile fatty acids. World Rabbit Sci., 12, 235-246.

Maertens L., Falcao-e-Cunha L., Marounek M. 2006. Feed additives to reduce the use of antibiotics. In: L. Maertens and P. Coudert (Eds.) Recent Advances in Rabbit Science. ILVO, Melle, Belgium, 259-265.

Marounek M., Vovk S., Skrivanová V. 1995. Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. Br. J. Nutr., 73, 463-469.

Michelan A.C., Scapinello C., Natali M.R.M., Furlan A.C., Sakaguti M.J., Vovk S., Skrivanová V. 2006. A note on the effect of caprylic acid on performance and mortality of growing rabbits. In Proc.: 7th World Rabbit Congress, Valencia, Spain, 437-444.

Simon O., Vilfried V., Scharer L. 2003. Micro-organisms as feed additives – probiotics. In Proc.: 9th Intern. Symp. on Digestive Physiology in Pigs, Banff, AB, Canada, 295-318.

Skrivanová V., Marounek M. 2002. Effect of caprylic acid on performance and mortality of growing rabbits. Acta Vet. Brno, 71, 435-439.

Skrivanová, V., Marounek M. 2006. A note on the effect of triacylglycerols of caprylic and capric acid on performance, mortality, and digestibility of nutrients in young rabbits. Anim. Feed Sci. Technol., 127, 161-168.

Strauss G., Hayler R. 2001. Effects of organic acids on performance and health of growing rabbits. World Rabbit Sci., 13, 17-28.

Wegener H.C. 2006. Use of antimicrobial growth promoters in food animals: the risk outweigh the benefits. In D.Barug, J. de Jong, A.K. Kies, M. W.A. Versteegen (Eds.) Antimicrobial Growth Promoters. Wageningen Academic Publishers, The Netherlands, 53-58.

Yamani K.A., Ibrahim H., Rashwan A.A., El-Gendy K.M. 1992. Effects of a pelleted diet supplemented with probiotic (Lacto-Sacc) and water supplemented with a combination of probiotic and acidifier (Acid-Pak 4Way) on digestibility, growth carcass and physiological aspects of weaning New Zealand White rabbits. J. Appl. Rabbit Res., 15, 1087-1100.

Zieremer C.J., Gibson G. 1998. An overview of probiotics, prebiotics and symbiotics in the functional food concept: perspectives and futures strategies. Dairy J., 8, 473-479.