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 B_{12}, 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	Energy retention	I	Ammonia production	R
Gut wall diameter	R	Gut energy loss	R	Toxic amine production	R
Gut wall length	R	Nitrogen retention	I	Alfa-toxin production	R
Gut wall weight	R	Limiting amino acid supply	I	Fatty acid oxidation	R
Gut absorptive capacity	I	Vitamin absorption	I	Faecal fat excretion	R
Faecal moisture	R	Vitamin synthesis	R	Liver protein synthesis	I
Mucosal cell turnover	R	Trace element absorption	I	Gut alkaline phosphatase	I
Stress	R	Fatty acid absorption	I	Gut urease	R
Feed intake	R = I	Glucose absorption	I		
		Calcium absorption	I		
		Plasma nutrients	I		

R: a reduction; = : no effect; I: an increase

(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. Insignificant 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. *toyo*i, *B. licheniformis*, *B. subtilis*) *Enterococcus* (*E. faecium*), *Lactobacillus* (*L.*

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[1]	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
			8	1%	60		
			9	0.15%	93	15.5% CF	Less favourable conditions (high density during several months)
			10	1%	95		
Maertens *et al.*, 1994	28-70 d	Paciflor (*Bacillus* CIP 5832)	11	0.01% (10^6 spores/g)	90	16% CF	Optimal housing conditions
			12	0.01% (10^6 cfu/g)	142	16% CF	Less favourable conditions
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. lichenjformis, B. subtilis*)	14	0.04% (1.28×10^6cfu/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% (8×10^{11} cfu/g)	27	12.5% CF	Experimental
Trocino *et al.*, 2005	35-70 d	Toyocerin (*B. cereus* var. toyoi)	16	0.02% (2×10^5 spores/g)	63 cages	41% NDF	Commercial
			17	0.1% (1×10^9 spores/g)	62 cages		
Esteve-García *et al.*, 2005	28 d	Toyocerin (*B. cereus* var. toyoi)	18	0.02%	15 × 5 cages	14.9% CF	Experimental
			19	0.05%	15 × 5 cages		
			20	0.10%	15 × 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.
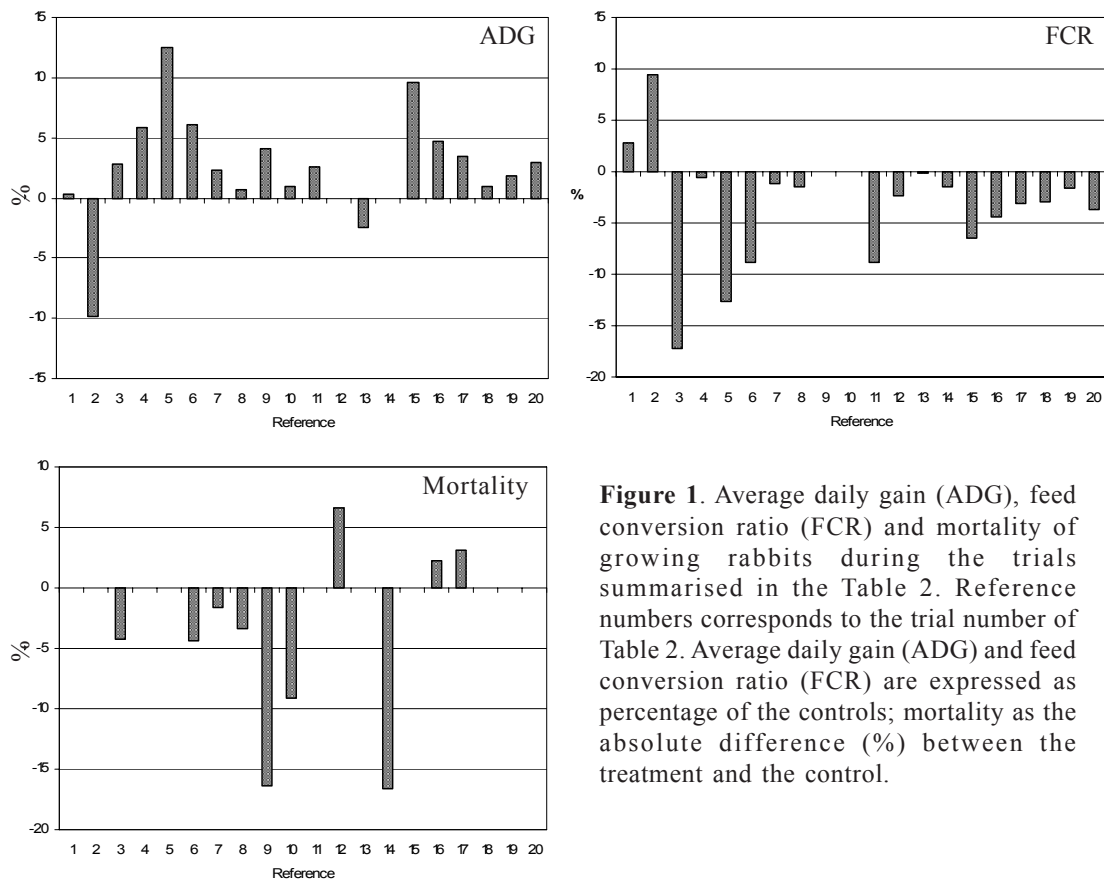
Figure 1. Average daily gain (ADG), feed conversion ratio (FCR) and mortality of growing rabbits during the trials summarised in the Table 2. Reference numbers corresponds to the trial number of Table 2. Average daily gain (ADG) and feed conversion ratio (FCR) are expressed as percentage of the controls; mortality as the absolute difference (%) between the treatment and the control.

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 cellulolytic 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.

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

*$P<0.05$

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.

Table 4: Effect of FOS addition on performances and mortality of growing rabbits from the trials.

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)	-[1]
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.

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 observed 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 (Choct, 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

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-gluccosidase, 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).

Table 5: Effect of organic acid addition in performances (differences in % of control group) and mortality (%control - %experimental) of growing rabbits on the trials.

Reference	Average daily gain	Feed conversion	Mortality
Hollister *et al.*, 1990	− 4.0% (40.1 *vs* 38.5 g/d)	+3.77% (3.77 *vs* 3.91)	− 7.2% (17.9 *vs* 10.7)
Scapinello *et al.*, 2001	+10.7% (28 *vs* 31 g/d)	− 3.9% (3.34 *vs* 3.21)	-[1]
Michelan *et al.*, 2002	+22.0% (27.5 *vs* 33.6 g/d)	− 14% (4.13 *vs* 3.55)	-[1]

[1] values not reported

The minimum inhibitory concentrations of organic acids for pathogenic microorganisms has been measured *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 Skøivanová 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, Skøivanová 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.

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