Strategies for the reduction of antibiotic utilization during rearing

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ABSTRACT: There are several management methods which have proved to be successful in reducing the disease pressure in rabbit production. All-in all-out management combined with an effective cleaning, disinfection and prevention is a primary tool to reduce the disease risks and by consequence both preventive as curative antibiotic utilization. A second tool is the use of minimal disease level reproduction stock. As it is more and more the habit in pig production, healthy and even animals with a near SPF status reduce the disease risks and lead to high-health-status farms where antibiotics have rarely to be used. Enteric troubles are the most frequent disease problem and responsible for an excessive use of antibiotics. Apart from some qualitative feed characteristics, know for their impact on enteritis risks (especially the carbohydrate complex), also quantitative aspects in early fattening stage are important to reduce enteritis risks. Finally, although there is a lot of discussion concerning their effectiveness, pro(pre)biotics and some other additives have proven to have some potential in reducing enteric problems. However, the whole chain (reproduction stock producers, breeders, feed companies, slaughterhouse and even veterinarians) have to be convinced and forced in a global strategy for minimal antibiotic use. Otherwise both preventive and curative use of antibiotics remains a too easy and even not very expensive way to handle the disease control in rabbit production.

Key words: Rabbits, Management, Nutrition, Antibiotic alternatives

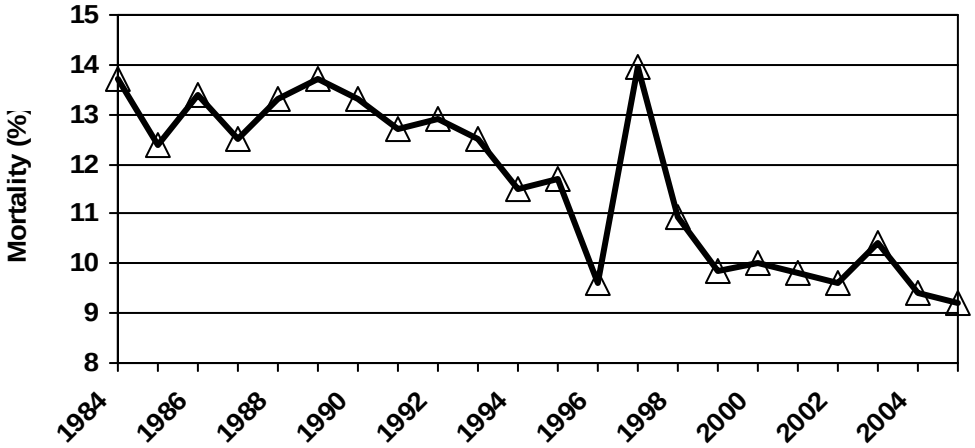
INTRODUCTION – In intensive animal producing units' large numbers of animals are housed in mainly closed houses (e.g. piglets, broilers, rabbits). This high density of animals leads to increased infection pressure and, especially in sensitive young animals, a high incidence of digestive disorders is observed. The ban of antimicrobial growth promoting (AGP) agents in the EC has increased these problems. Zinc bacitracin was the most used AGP in rabbit feed. Moreover, even the therapeutic treatment with antibiotics of food-producing animals, has been viewed critically because of their impact on the development of resistant bacteria and/or residues that compromise human health.

Although a lot of discussion exists between the use of antibiotics in food-producing animals and human health, we can not deny the growing public concern for "healthy" produced food. Apart from legislation, more and more the large food retailers and food service companies are the "driving engine" how animal products have to be produced. Some examples are the exclusive distribution of free range produced eggs by the Dutch warehouse "Albert Heyn" or the Belgian "Colruyt" or the declaration in The Netherlands to sell from 2009 on, only meat from pigs that were strayed castrated.

Another example is in the U.S. where Tyson Foods only sells fresh meat of chickens that is produced without antibiotics.
These examples clearly show that also rabbit meat production has to change to production systems taking into account consumers' confidence without e.g. systematic treatments against pasteurellosis or the use of medicated feeds mainly to overcome digestive disorders. In poultry and pig industry, increasing standards of animal hygiene are applied to enable production models with a low antibiotic use. Although also efforts are done in rabbit meat production, due to e.g. the smaller production units, prevention programs are more difficult in rabbits. Furthermore, the etiology of enteropathies, which are responsible for a significant mortality and morbidity of rabbits after weaning, is often unclear because causes are frequently multiple and some pathogenic agents have not yet been identified (Licois *et al*., 2006).
There exist a lot of strategies to achieve the aforementioned goal. Besides improved management techniques, other strategies to reduce the necessity of antibiotics as the use of high-health animals, nutrition and feeding strategies and alternatives to in-feed antibiotics will be discussed in this paper.

1. MANAGEMENT TOOLS – Rabbit production systems have changed dramatically since the introduction of artificial insemination in the late eighties. From an individual management of each female, group (batch) systems were developed. The advantage was not only to improve the management in the farm but also to develop a better management in the total production chain (farmer, feed company, slaughterhouse …) and allowing to facilitate traceability procedures (Cavani and Petracci, 2006).
However, batch handling of females has made it possible to develop real "all in all out" production systems. Such systems are recognized in all animal productions as a basis to overcome and to control disease problems and by consequence to reduce the use of antibiotics. In rabbit production, the "single batch system" or in French "La bande unique" has known increasing interest and in 2005 already 81% of the commercial farms in France followed this management system (Azard and Lebas, 2006).

Figure 1. Mortality after weaning in french rabbitries



The single batch system perfectly fits with an "all in all out", allowing total cleaning and infection every 11-12 weeks when using a 42 days rebreeding system. With increasing introduction of the combination "single batch + all in all out", mortality after

weaning decreased from 13-14% before 1990 till less than 10% after 2000 (Figure 1). From a scientifically viewpoint, however, we may not exclusively attribute this significant reduction in mortality to the aforementioned change in rabbit management. Nevertheless, this positive trend goes hand in hand with the increasing use of "all in all out" and is observed in spite of the enteropathy problems and the ban of antimicrobial growth promoting agents.
Another example to illustrate the effect of a clean environment is taken from our experimental unit. When we compare the results of the same batch of weanlings housed in an "all in all out" unit with the littermates housed in a continuously occupied unit, not only the mortality was much lower (1.8% vs 12.1%) but also daily weight gain of the surviving rabbits was 2.3 % higher (43.4 vs 42.4 g) (Maertens and De Groote, 1992).
The practical all in all out system requires at least 2 buildings with the same equipment and is called DUO system. Cages are adapted so that they can be used for reproducing females and for fattening. At weaning the females (already at least 25 days pregnant) are transferred to the 2nd building while the young remain till the end of the fattening period. All fatteners are delivered to the slaughter house on the same day and some days are available to clean and disinfect the empty house. This house is now ready to accept again the females of the other building.

2. SPF OR MINIMUM DISEASE LEVEL RABBITS – Also in other animal productions (e.g. pigs) successful efforts are done to produce breeding stock free from a number of specified pathogens. Specified Pathogen Free rabbits (SPF) can be obtained by hysterectomy but also by following a strict prophylactic program in a well-controlled environment combined with very strict hygienic measures (Coudert *et al*., 1988). However, the use of SPF rabbits for commercial rabbit meat production is not economically because of the large input to maintain this status but also because these animals are very sensitive to pathogens when introduced into more conventional housing systems.

Table 1. Comparison of the zootechnical results before and after the change of sanitary status

	Conventional status (1985-1988)	MDL status (1989-1993)	Difference (%)
Pregnancy rate (%)	68.5	74.2	+ 8.3
Born alive	8.13	8.22	+ 1.2
Mortality before weaning (%)	15.5	11.2	- 28.1
Mortality after weaning (%)	13.0	1.72	- 86.7
Weaning weight (g)	674	764	+ 13.4
Daily weight gain (g)	37.2	41.8	+ 12.5
Weight at 11 weeks	2406	2735	+ 13.7
Feed conversion (economical)	3.5	2.8	- 19.4

Adapted from Hendrickx *et al*. (1994)

The elimination of the main specific pathogenic agents leads to farms called "Minimal disease level (MDL) or "High-health-status farms" (HHF). Already 15 years ago, the positive effects of the transfer from a conventional status to an MDL status were clearly demonstrated (Hendrickx *et al*., 1994). In Table 1, the zootechnical performances before and after the change of the sanitary status are presented. MDL rabbits were obtained by

aseptic cesarean from the conventional population and artificially reared. The main goal, to reduce the mortality after weaning, was dramatically achieved and decreased from 13.0% till only 1.7%. Moreover, this result was obtained without the use of any antibiotic treatment while before the transfer to the MDL status antibiotic treatments were frequently used. Not surprising is that also the other zootechnical performances were much better after the change of the sanitary status. Performances of the does increased in terms of pregnancy rate but were also observed in the weaning weight of their kits (+13.4%). Subsequent weight gain in the fattening period was more than 12% higher and resulted, combined with the reduced mortality, in a 20% more favourable feed efficiency.

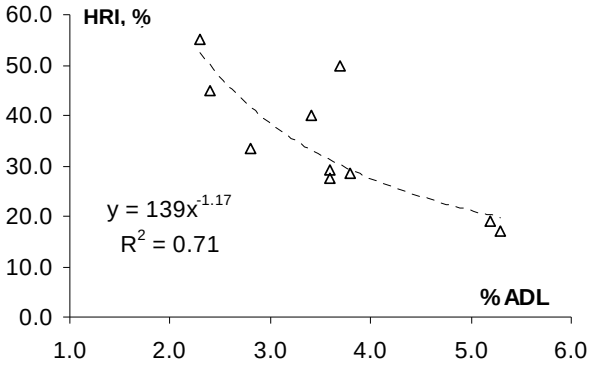
When the sanitary status of the farm or experimental unit is free from highly pathogenic agents, it is possible to achieve low mortality levels without the use of any antibiotic treatment. For example in our experimental unit, before the introduction of enteropathy, post weaning mortality was lower than 5% (e.g. Maertens *et al.,* 1997). More recently results with very low mortality were published and obtained without antibiotic treatments in the diet or drinking water (e.g. Garcia-Palomares *et al*., 2006; Alvarez *et al.,* 2007). However, in enteropathy infected units mortality can reach unacceptable high levels without the use of antibiotics (Licois *et al*., 2006; Maertens *et al*., 2005, Gidenne *et al*., 2005; Feugier *et al*., 2006).

These results illustrate that to achieve a reduced use of antibiotics in rabbit production, a key role is in the hands of selection units since the majority of commercial farms introduce hybrid stock. The introduction (or renewal) of healthy reproduction stock is of basic importance to come to a high-health-status farm. In many cases, a necessary complete depopulation of the farm and the introduction of newly, guaranteed healthy reproduction stock together with increased hygienic measures, allowing to maintain this status, will be necessary as already long-time suggested (Peeters, 1988) and still recommended in case of e.g. endemic pasteurellosis (Coudert *et al*., 2006). New tools that allow screening the newly acquired rabbits are available for several pathogens (Licois, 2006) and helpful to reach the goal of a high-health-farm status.

3. NUTRITION TOOLS – It is already long-time known that fibre supply plays a major role in the prevention of digestive troubles (Lebas, 1980). However, due to better analytical methods to estimate fibre fractions and intensive research, the role of the different fractions was much better defined (Gidenne, 2003).

Lignins and to a lesser extend also cellulose remain largely undigested, because of their polyphenolic structure that is not hydrolysed by the bacteria in the rabbit caecum. Lignins play a dominant role in the transit time in the gut, and increasing levels are associated with a significant reduction of the digesta retention time. In several experiments it has been shown that there exist a relationship between the dietary ADL level and the mortality due to enteritis (see review Gidenne, 2003). This relationship was even more pronounced when the health risk index (HRi) was confronted with the dietary ADL level (Fig. 2).

Based on these data a dietary level of 5.5% ADL is recommended after weaning and minimum 5.0% in the following growing period to prevent against digestive troubles (Table 2). The effects of cellulose intake are less important than for ADL, regarding the decrease in retention time and favouring the health status. Globally, the ADL requirement for the growing rabbit can be assumed as to 5 to 7g/d, and that of cellulose from approximately 11 to 12 g/d (Gidenne and Garcia , 2006).



ADL = Acid detergent lignin (Van-Soest sequential procedure).

HRI = Health risk from digestive trouble = Mortality + morbidity rate by diarrhoea, measured from 28 to 70d of age, on at least 40 rabbits/diet (Data for 10 diets ranging from 14 to 20% ADF level; references: Gidenne, 2003).

Figure 2. Reduction of digestive troubles incidence according to dietary lignin (Gidenne, 2003).

Hemicellose and pectins are considered as digestible fibre because their digestibility is around 30 and 70%, respectively. The retention time in the caeco-colic segment is relatively short (8-12h), therefore these rapidly fermentable cell-wall polysaccharides play a key role in the rabbit digestive processes. They are important to obtain a correct fermentative activity both in the small intestine and the caecum. It has been shown that the uronic acids (main part of the pectins) modulate the fermentative activity in the caecum and the caecal pH. By consequence a sufficient dietary content of these digestible fibres is necessary besides the indigestible fraction for optimizing digestive health (Gidenne, 2003). However, the dietary digestible fibre content has to be in balance with the other fibre fractions and at a too high content health risks increase. Therefore it is recommended to remain below a dietary ratio dig. fibre/ADF under 1.3.

Table 2. Some recommended dietary levels in a complete feed to optimize digestive health

As % of a diet with 90% DM	Young rabbits (4 - 7 weeks)	Growing (7 - 10 weeks)
Crude protein	<16.0	< 16.0
NDF	≥ 31.0	≥ 27.0
ADF	≥ 19.0	≥ 17.0
ADL	≥ 5.5	≥ 5.0
Dig. fibres/ADF	≤ 1.3	≤ 1.3
NDSF	12.0	
Particles > 0.3 mm		>21.0

Adapted from Gidenne and Garcia, 2006

The importance of **rapidly fermented polysaccharides** (determined as neutral detergent soluble fibre, NDSF) was also recently stressed by a Spanish team (Gómez-Conde *et al*., 2004; Gidenne and Garcia, 2006). They showed that a too low level (8%) of this fraction reduces intestinal health (Table 3).

Starch has been supposed to be a factor predisposing to the development of undesirable flora in rabbits. Recent studies, however, have demonstrated that the starch intake and

its incidence on digestive health are of minor importance compared to the cell-wall polysaccharides (see review Gidenne and Garcia, 2006).

Protein requirements are high in young animals not only for body growth, but also for intestinal mucosa development and renewing. However, the replacement of fibre by

Table 3. Effect of dietary NDSF level on digestive parameters at 35d, and mortality in 25d weaned rabbits (Data of the team of Gómez-Conde *et al*., 2004 adapted by Gidenne and Garcia, 2006).

Dietary NDSF[1] level, % (as fed)	12	9	7	P level
Jejunum morphology and functionality (35d)				
Villi length, µm	721[a]	567[b]	492[c]	0.05
Crypt depth, µm	89[b]	115[a]	113[a]	0.05
Saccharidase activity (U/mg tissue)	8500[a]	7100[b]	5400[c]	0.05
Immune response in *lamina propria* (35d)				
CD4+, %	35	33	26	NS
CD8+, %	21[b]	27[b]	31[a]	0.05
C. perfringens, %[2]	8[b]	6[b]	19[a]	0.05
Mortality 25-60 d, %	5.3[b]	8.5[ab]	14.4[a]	0.05

[1] Neutral detergent soluble fibre according to Hall *et al.* (1997); [2] Frequency of detection in the ileum or caecum

protein and resulting in an excess of protein leads to increased risk for diarrhoea (Garcia *et al*., 2004). A hypothesis to explain it could be a higher availability of substrates for microbial growth, with prevalence of pathogenic species, when animals are fed with high protein diets (Carabaño *et al*., 2006). Accordingly, a lower ileal flux of protein (-20%) was observed with the low protein diet (16.2% *vs* 18.9%) parallel with a lower mortality (1.3% *vs* 7.7%) and decreased detection frequency of *Clostridium perfringens* in the ileum (Chamorra *et al*., 2005). This event would be more important in young rabbits due to the unachieved digestive maturation.

Dietary factors have large implications in the development of the immune response. In this respect the profile of dietary fatty acids (ω3/ω6 ratio) lead to improved immune competency and health status in several species and could be of interest in rabbits (Fortun-Lamothe and Bouillier, 2007). Diet has also an important role in the colonisation of the intestine. However, the gastrointestinal microbiota is a complex community that remains largely unknown. New techniques are hopeful to provide better knowledge about the role of individual bacteria in this community and to establish the conditions for healthy microbiota (Carabaño *et al*., 2006).

Besides qualitative aspects, quantitative aspects of feeding have proved to be helpful to overcome losses due to diarrhoea. Based on some recent studies and the success under practical conditions, many farms do no longer feed weanlings *ad libitum*. A reduction of the feed intake with at least 25% has proven to be very helpful to overcome enteritis problems between the ages of 5 – 8 weeks. Mortality rate was only the half in restricted fed rabbits compared to those fed free pellets (Gidenne *et al.*, 2003). On intensive farms increasing use is therefore made of automatic feeding systems which allow distributing a restricted quantity of feed in relation to the age of the growing rabbit.

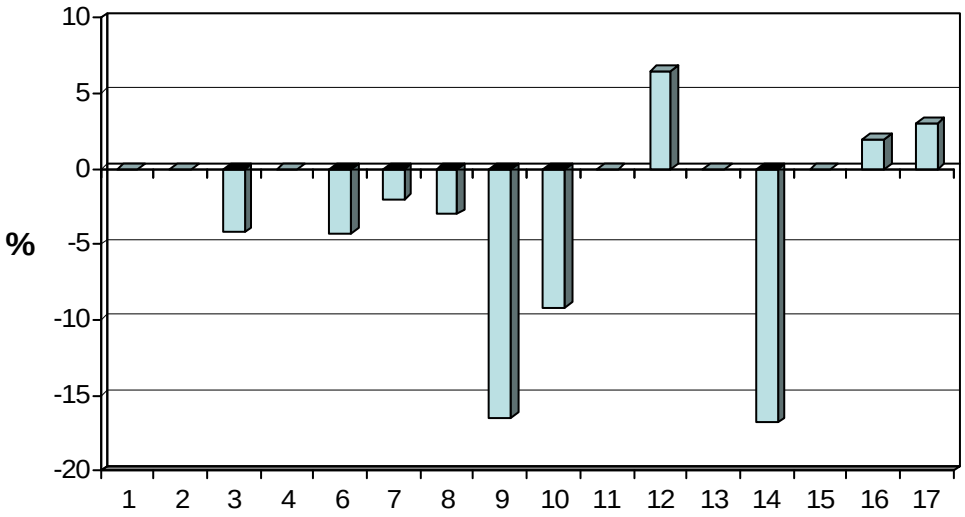
An indirect method to restrict the feed intake level is to restrict the water intake. Rabbits have only a quite small stomach which limits a high water and feed intake during a short time. Therefore, when the water distribution is limited till 2.5-3 (continuous) hours per day, feed intake is only 70% of the *ad libitum* intake. Under such conditions, both in experimental conditions as in farms, positive results were obtained to reduce the enteritis problems and the losses due to diarrhoea (Boisot *et al*., 2003; 2004).

4. ALTERNATIVES TO IN-FEED ANTIBIOTICS – There are a number of non-therapeutic alternatives for in-feed antibiotics including probiotics, prebiotics, synbiotics, (in)organic acids, plant extracts, enzymes and immune modulators. In this chapter an overview of the results in rabbits, focussed on the health status, with these alternatives is intended.

Probiotics by definition are live micro-organisms which beneficially affect the host animal by improving its intestinal microbial balance. Probiotics are reported to exert their beneficial effects in several ways including balancing of colonic microbiota and enhanced immune response (Fortun-Lamothe and Boullier, 2007). Most micro-organisms used in probiotics are strains of gram-positive bacteria of the genera *Bacillus*, *Enterococcus*, *Lactobacillus*, *Streptococcus* or yeast. 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.

There exist a reasonable number of experimental trials with probiotics in rabbits. However, only a very few number were published in refereed journals and most of the results were presented at the occasion of congresses. The results of these trials with fatteners are summarized in Figure 3. Mortality is presented as the absolute difference; in percentage points, between a treatment and the corresponding control group (see review Falcao-e-Cunha *et al*., 2007). Although mortality was reduced in the majority of trials (7 positive, 6 null and 3 negative results), only in 2 of the trials the effect was significant.

Figure 3. Overview of the effect of probiotics on mortality in weaned rabbits (difference= % mortality in probiotic diet - % mortality in control diet)



References (see Falcao-e-Cunha *et al*., 2007)

Inconsistent results with probiotics are in fact not surprising given the complexity of the gut ecosystem, where the probiotic is expected to have its effects. Therefore, studies regarding the direct relation between probiotic addition and digestive health in rabbits are necessary to improve the optimal conditions. Moreover, for rabbits the probiotic micro-organisms must be able to withstand the pelleting conditions and storage of the feed.

Prebiotics are another possible alternative to antibiotics. They are usually defined as non-digestible food ingredients that stimulate selectively the growth and (or) activity of potentially health-enhancing intestinal bacteria. Prebiotics generally refer to carbohydrates, mainly oligosaccharides that target the beneficial bacteria of caeco-colic microflora. The main commercial oligosaccharides are fructo-oligosaccharides (FOS), α-galacto-oligosaccharides (GOS), mannan-oligosaccharides (MOS) and 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. Firstly, they can prevent the adhesion of pathogens to the mucosa, by competing with its sugar receptors, and secondly they can stimulate the gut immune system**.**

As for probiotics, the reported effects of prebiotics on rabbit performances and caecal pH or VFA levels are unclear (see review Maertens *et al.*, 2006). However, mannanoligosaccharides (MOS) have shown to have potential to prevent pathogens from colonizing the alimentary tract. Many pathogens have fimbriae which specifically attach to the mannose residues of intestinal cell receptors, and by connecting to MOS they 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.

Another type of fructans namely inulins, reduced caecal pH and increased volatile fatty acids concentrations (Volek *et al.*, 2005) or a higher butyrate proportion (Maertens *et al.*, 2004). Moreover structural changes in the gut architecture were observed (Alves *et al.*, 2003). This fits with the major aim of the use of prebiotics in rabbit feed, that is to create conditions in the caecum that are not favourable to *Clostridium* development.

Organic acids are also considered as an alternative to antibiotics and namely in pig feeds they already have considerable success. However, in rabbits there exist only few studies and the results are inconsistent (Falcao-e-Cunha *et al*., 2007). On the other hand, a group of Czech researchers have studied intensively the effects of medium-chain fatty acids in rabbits. In a study of Skřivanová 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, testing the medium-chain fatty acids esterified in triglycerides, these results were confirmed, i.e. a significant reduction in post-weaning mortality (Skřivanová and Marounek (2006).

Enzyme addition in rabbit feeding has reached more attention during the last years, especially in young animals having an incomplete digestive system. When starter diets were enriched with a mixture of beta-glucanase, beta-xylanase and α-amylase mortality decreased over the whole fattening period (Gutiérrez *et al.*, 2002). The beneficial effects of the addition of an enzyme complex, when the incidence of intestinal disorders and mortality are of relevance, were confirmed and explained through the decrease of the amounts of nutrients reaching the fermentative area (Calchaldora *et al*., 2004; Garcia *et al.*, 2004).

Plant extracts are considered due to their specific bio-active components also as an alternative and used in other animal productions (e.g. etheric oils, garlic, thyme,…). Specific *in-vitro* antimicrobial activity has been demonstrated for some herbs. However, to our knowledge no publications are available indicating that their use favours intestinal health and by consequence reduces mortality in rabbits. Hydrolysable tannins seem to have some potential especially to overcome protein overload in the gut (Maertens and Štruklec, 2006). However, the results are not consistent as it is the case with most of the in-feed alternatives.

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 (Falcão-e-Cunha *et al*., 2007).

CONCLUSION – Pathogenic agents cause large losses in rabbit production and thereby antibiotic treatments are extensively used. Because of the lack of efficient alternatives (e.g. vaccines) a global approach including non-nutritional and nutritional measures is necessary to reduce the use of antibiotics. An eradication program based on an increased management is a first and necessary step. The use of reproduction stock free of some heavy pathologies and reared in high-health conditions has proved both in experimental and in farms to lead to reduced mortality. The importance of dietary factors and especially the dietary fibre level and quality on rabbits' health status has been strongly evidenced. Diets adapted to the age and taken into account the sanitary situation of the farm are helpful to overcome digestive disorders. A wide range of feed additives are available which claim positive effects on intestinal health. Especially in rabbits most of these claims are based on empirical trials and results are not consistent. However, because of the complexity of rabbits' digestive system, advances in the fundamental modes of action have to lead to designed alternatives. Also, a combination of two or more of these alternatives, as in symbiotics, is still an opportunity to be fully explored.

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