SAFE ALTERNATIVE ADDITIVES TO ANTIBIOTICS IN RABBIT NUTRITION:
PROBIOTICS AND PREBIOTICS

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SUMMARY- Rabbit meat is interesting for its dietetic and nutritional characteristics and bio-security for
the consumer. In addition, the consumer as a product of high quality already accepts it. With
intensification of production systems and concurrent exposure to different stressors, antibiotics at sub-
therapeutic levels, have been widely introduced to rabbits as growth promoters for long time. Recently,
evidences on the increased health risk to people who consumed the products they contain urged the EU
and other countries to ban the usage of sub-therapeutic levels of antibiotics as antimicrobial growth
promoters, which in turn, forced the scientists and rabbit producers to seek for other natural, safe,
reliable, and economic additives serve the same goals achieved by antibiotics.
This paper reviews in short the rabbit gut ecosystem, and in more details the effects of probiotics, AND
prebiotics as alternatives to antibiotics in growth performance and health status of the rabbit.

Key words: Rabbit, ecosystem, antibiotics, probiotics, prebiotics, growth performance, health and safety.

INTRODUCTION

Rabbit meat for its dietetic and nutritional characteristics is accepted by the consumer as a product of
high quality already; it is lean and its lipids are highly unsaturated (60% of the total fatty acids), rich in
protein (20-21%) with high biological values amino acids, poor in cholesterol and sodium, and rich in
potassium and magnesium. The bio-security of rabbit meat for the consumer derives from the absence of
zoonosis and drug residues (Dalle Zotte, 2000).

Following intensive production systems (high genetic make-up breeds, high density of breeding unit,
developing feeding plans, early weaning, …) with concurrent presence of one or multi-factorial stressors
(environmental, nutritional, managerial,…), this cross-talk leads to the introduction of growth promoter
agents to maximize economics of rabbit meat production. They include chemicals (antibiotics, hormones,
copper compounds and others) or organic additives (probiotics, prebiotics, organic acids, enzymes,
essential oils, and others). For more than 50 years, antibiotics as antimicrobial growth promoters (AGPs)
have been applied in animal nutrition in an attempt to suppress/enhance the growth of
pathogenic/favorable bacteria of the gastro-intestinal tract, with acceptable results for the meat producer,
but not for the consumer. Recent evidence on the development of resistance in zoonotic organisms of
animal origin and consumers’ claims for safety animal foods urged the EU and other countries to ban the
use of the sub therapeutic levels of antibiotics as AGPs in animals. The ban went into force at January 1st
2006. After a short review of the rabbit's gut microflora, this review will pay attention to additives that
favor better rabbit gut microbiota with special emphasize on their effects on growth performance and
health status as safe, reliable and efficacious alternatives to antibiotics in rabbit nutrition.

RABBIT GUT ECOSYSTEM

As recently reviewed by Fortun-Lamothe and Boullier (2004), the development of the intestinal
microbiota is a gradual process, rabbit gut microbial colonization begins after birth. Bacteria are the main
constituent of the gut microbiota in rabbit, communities of archa (22% and 12% at 28 and 70 days of
age, respectively), protozoa and yeasts (Saccharomyces guttulata represents almost 100% of the yeast
inhabitant of the gut; Hullár et al., 1996). Streptococcus spp. and Escherichia coli, reach a maximum level
at the 2nd or 3rd week of age and then decreased to be residual or absent after weaning. The strictly
anaerobic, non-sporulating bacteria, especially Bacilli (Bacteroides) dominate the digestive flora in every
segment of the intestine. Sporulating bacteria (Clostridium, Endosporus and Acuformis), 100 to 1000
times less numerous than the Bacteroides. The absence of the genus Lactobacillus and anaerobic fungi
in the rabbit flora is original. When intake of solid food begins, fibrolytic flora increases slowly, which
through microbial fermentation in the caecum provide 12-40% of the rabbit's maintenance energy requirement as VFAs, these flora, also have the ability to synthesize B-complex and K vitamins, and through the process coprophagy (re-ingestion of soft feces) improve the nutritional value of feed (Cheeke, 1987). Although, not favor are the toxic substances including amines, phenols and indols, besides ammonia as a result to the caecal flora’ proteolytic and ureolytic activities (Gidenne, 1997). As a stress factor will affect the rabbit delicate gut eubiosis (balance between beneficial vs. pathogenic bacteria), a profound disturbance in rabbit performance well occur, as rabbit is extremely sensitive to digestive disorders, especially around weaning period. The mortality from enteritis may reach up to 50% of live rabbits (Peeters et al., 1995). Antibiotics and other feed/water supplements have been intensively studied around weaning to manipulate digestive dysfunction and improve performance traits.

ANTIBIOTICS

Antibiotics have been used in rabbits for therapeutic, prophylactic and as antimicrobial growth promoters (AGPs). The latter imply that antibiotics modify the gut flora, suppress bacterial catabolism and reduce bacterial fermentation. These changes lead to an improved health state and increased nutrient availability for the animal, thus increasing growth performance (reviewed by Pinheiro et al., 2004a).

Antibiotics always are not a magic as growth promoter for rabbits; results sometimes were encouraging (Richard et al., 2000, using Zn-bacitracin, Szábo et al., 1988, using Nitrovin and Skřivanová et al., 1999, using virginiamycin and salinomycin) and sometimes were not (Abu-El-Zahab et al., 1992 and Pinheiro et al., 2004a, using Zn-bacitracin). May be the circumstances of each study have a potential effect of the results recorded. Good management, sanitary and feeding practices, poor response to antibiotics is expected.

The effect of antibiotics on rabbit caecal microbial population was not done before, as reported by Pinheiro et al., (2004a). They observed that concentration of different bacterial population on caecal content, decreased significantly in growing rabbits with Zn-bacitracin feeding, where coliforms population decreased in half and total bacteria count in 26%, while Enterococci were reduced to non detectable levels

Recently however the effects of antibiotics on the development of resistant bacteria in both animals and humans, and associated risks to human health, have been the subjects of controversy. It is well documented that antibiotics included in the diet do select for resistance not only in pathogenic bacteria but also in the endogenous microflora of exposed animals (Fonseca et al., 2004). Fear of possible residues in animal meat and contamination during processing and emergence of antibiotics-resistant bacteria forced the EU and other countries to ban the use of the sub therapeutic levels of antibiotics as AGPs in animals. The ban went into effect at January 1st 2006.

PROBIOTICS

Many definitions have been suggested to describe the term probiotics “for life”. More recently, after revising previous definitions, Schrezenmeir and de vrese (2001) concluded that a probiotic is” a preparation of or a product containing viable, defined microorganisms in sufficient numbers that in a compartment of the host and by that exert beneficial health effects in the host”.

In contrast with antibiotics, probiotics objective is not to destroy pathogenic bacteria, but to exercise a barrier effect against pathogens by preventing their development and colonization in order to secure optimum utility of the feed (Maertens et al., 1994). In other words; they promote stabilize eubiosis by competitive growth against harmful microorganisms, reducing the intestinal pH with production of lactic acid and encouraging digestion by producing enzymes and vitamins, these functions strengthen the animal’s own non-specific immune defense (Kustos et al., 2004). Some other probiotic benefits were earlier reported by Fuller (1989): decrease carcass contamination, decrease ammonia and urea excretion, improve mineral absorption, lower serum cholesterol and lower skatol, indole, and phenol (odor compounds).
The most common probiotic preparations include the beneficial bacterial strains of Lactobacilli, Streptococci, Bacilli and Biofidobacteria, and yeasts (Saccharomyces strains) or their mixtures. The characteristics of ideal probiotics include: Be of the host origin, non-pathogenic, withstand processing and storage, resist gastric and bile acids, adhere to the epithelium or mucus, persist in the intestinal tract, produce inhibitory compounds (against pathogens), modulate immune response, and alter microbial activity (Simmering and Blaut, 2001).

Most studies on the effect of probiotics on rabbit performance are undertaken around critical weaning period, which is characterized by unstable bacterial ecosystem due to changing from maternal to solid feeding with immature defense system against pathogens, the results are always inconstant under the same conditions, but generally are encouraging.

**Lactic acid bacteria containing commercial products:** Lactobacilli (lactic acid producing bacteria) i.e. Streptococcus faecium and Lactobacillus acidophilus are able to produce lactic acid from carbohydrate and are resistant to acidity as a result, while acid is fatal to other bacteria (e.g. E. coli) (Gippert et al., 1992). Occurrence of multiple organic growth promoters including a probiotic preparation represents an approach for further associative effect, since in most cases, the eminient response to a probiotic is linked to sanitary status alone. In this connection, Lacto-sacc® composed of microencapsulated S. faecium, and L. acidophilus, yeast culture, Saccharomyces cerevisiae strain 1026, dried A. niger fermentation extract and enzymes. Variant results were recorded when applying Lacto-sacc in rabbits; had no effect on growth performance when used at 0.5, 1.0 or 1.5 g/kg diet (Ismaiel et al., 2004), reduced incidence of enteritis about 50% with administration of 1.25 g Lacto-sacc/ L water, but had no effect on weight gain (Hollister et al., 1989). Marginally decreased mortality and increased weight gain when added at the feed at 1 g/kg feed (Gippert et al., 1992). Significantly increase weight gain and improved feed conversion ratio at the rate of 1 g/kg rabbit feed (Yamani et al., 1992). When added under higher environmental temperatures at high rate (2 g/kg feed), significantly improved weight gain and favorably decreased mortality in manner, equally to the antibiotics, avoparcin, flavomycin or Zn-bacitracin (Abdel-Samee, 1995). Also, Acid-Pak-4-Way, a mixture of S. faecium, and L. acidophilus, A. niger, B. subtilis, citric and sorbic acids, sodium citrate, electrolytes and feed enzymes reduced the haemolytic E. coli, increased survival rate and decreased the incidence of enteritis when added at the rate of 5 g/L (Hollister et al., 1989). Latter, Yamani et al., (1992), reported that 0.5 g/ L was effective to improve growth performance of rabbits compared with the control or a high level of 2.0 g/L. Lact-A-Bac® is a dried Lactobacillus acidophilus (0.8 billion CFU/g), when compared at levels 0.1 or 0.2% with the antibiotic, Stafac®20 (contain 2% virginiamycin), comparable growth performance was recorded between the supplements (El-Adawy et al., 2000), while at the rate of 0.5 g/kg rabbit diet, improved significantly weight gain and feed conversion, moreover, increased Lactobacilli and cellulolytic bacteria and decreased caceal NH3 (Amber et al., 2004).

**Streptococcus spp.** post-weaning enteritis mortality decreased from 22.4 to 6.3 %, when streptococcus faecium included in rabbit diet as approved by Maseoro et al., (1980; see Gippert, et al., 1992), also 50% reduction in mortality rate, associated with 3% increase in growth weight when fed growing rabbits a diet supplemented with 1.6 g dried Streptococcus faecium; where 1.0 g contain 120 million living acid bacteria (Lacza and Gippert,1988).They attributed the favorable effect of Streptococcus faecium on rabbits that it prevents the harmful bacteria to penetrate, to stick and to multiply in the intestinal flora. It decreases the pH value in the digestive tract hindering with primarily the multiplication of the E. coli, it promotes the multiplication of the cellulitic bacteria. They added that the precondition of the practical use of the product is that it can be mixed into the rabbit feed without damage.

**Saccharomyces cerevisiae:** Performance of growing rabbits were significantly improved under less favorable housing conditions, when fed diets supplemented with Biosaf® (a live yeast of Saccharomyces cerevisiae) at the rate of 0.15 or 1.0% to provide 7.5 X 10⁶ and 5 X 10⁷ CFU/g, respectively, while the response to such additive was of no value under optimal housing conditions as reported by Maertens and De Groote, (1992) who supposed that Saccharomyces cerevisiae is able to transit under a viable form all along the digestive tract, carrying the E. coli fixed on their membrane. Also, comparing the probiotics, Diamond V; a yeast culture (Saccharomyces cerevisiae; 2 g/ kg diet) or Lact-A-Bac; dried Lactobacillus
**Saccharomyces cerevisiae** (0.8 billion CFU/g; at the rate of 0.5 g/kg diet) and the antibiotics, flavomycin or Zn-bacitracin at level of 100 mg/kg diet). Diamond V showed, significantly, the best growth performance, the other supplemented were equal to the control. Both, probiotics containing supplements showed the highest microbial count (streptococci, lactobacilli, lipolytic bacteria, yeast and actinomycetes) as reported by Soliman et al., (2000). While, Jerome et al., (1996) reported that neither live yeast (*Saccharomyces cerevisiae*, Sc 47; 10⁶ CFU/g feed) nor oxytetracycline (200 ppm), individually or in a combination improved growth performance of rabbits.

**Bacillus spp.** the most dominates of the rabbit intestine, *Toyocerin®* (concentration: 1 x 10⁵ *Bacillus cereus* var. *toyoi* spores/g) when added at the rate of 200 ppm (2 x 10⁵ spores/g) or 1000 ppm (1 x 10⁶ spores/g), significantly improved live weight gain and feed conversion and lowered morbidity, but had no effect on mortality or sanitary risk as reported by Trocino et al., (2005). The same authors reviewed the response of rabbits to dietary supplementation with various species of *Bacillus*. In comparison with other probiotics, *Bacillus* supplementation is easier to carry out from a technical point of view, due to the resistance of the spores during storage and feed processing. Competition has been shown to exist between *Bacillus spp.* and the pathogenic flora at the gastro-intestinal level, which could help in maintaining a positive flora and good health condition. However, the inclusion of *B. subtilis* did not improve growth or health status of growing rabbits in some previous works (Cristofalo et al., 1980 and Lambertini et al., 1990). While, when *B. subtilis* was associated with *B. licheniformis*, growth rate, and feed conversion improved (Zoccarato et al. 1995, and Bonanno et al., 1999) and mortality was substantially reduced (Bonanno et al., 1996). On of earlier works carried out by Hattori et al., (1984) showed that increasing *Toyocerin®* level (1 x 10⁶, 1 x 10⁵ and 5 x 10⁵ *Bacillus toyoi* spores/g feed) caused a significant reduction and complete prevention of diarrhea in rabbits, and a remarkable favorable effect on live weight gain. Yet, a significant decrease in *E. coli* was found in rabbits consuming 1 x 10⁵ and 5 x 10⁶ spores/g feed. spores of *Paciflor®* (Bacillus CIP 5832, 1.0 x 10⁶ CFU/g); a strain with microbiological characteristics similar to those of *Bacillus subtilis* when included in rabbit diet (0.1%), increased daily weight gain, while, mortality rate from diarrhea decreased by 60%, the response to *Paciflor®* was higher when growth rate was limited because heat stress or low weight at weaning (de Blas et al., 1991). In contrast, the trial undertaken by Vörös and Gaal, (1992) came to that 0.01% *Paciflor®* had no effect on daily weight gain, feed conversion or mortality rate of rabbits. Again, Maertens, et al., (1994) reported that 0.01% *Paciflor®* had no effect on live weight gain, feed conversion, and VFAs, pH caecum or NH₃ production. Another, is *BioPlus 2B®* (combination of spore-forming bacteria of *Bacillus licheniformis* and *Bacillus subtilis*), when fed to rabbits (400 mg/kg diet; corresponded to 1.28 x 10⁵ CFU after pelletization) it had no further effect on increased live weight gain or efficiency of feed utilization, but showed substantial, favorable effect on decreasing mortality and sanitary risk (morbidity and mortality; Kustos et al., 2004).

Conclusively, we can rely on probiotics, firstly, as an enhancer for health status more than growth enhancer, where they improve the resistance to pathogens and decrease sanitary risk. Yet, results in most cases, indicate that probiotics are not less effectual of antibiotics in improving zootechnical performance. The no response with some cited studies may be due to the type of probiotic included, we see that rabbit is of peculiar gut microflora, while commercial probiotics may fit other livestock rather than rabbits. More critical, the conditions of study under which a probiotic employed. Also, how much of the probiotic involved in a diet stand pelletization and variation in different gut compartment to exert beneficial role in the target organ. All these and others are limiting factors on the response of rabbits to probiotics supplementation.

**Safety and toxicity:**

European Commission approved in 2001 the probiotics, *Bacillus cereus*, *Bacillus cereus* var. *toyoi* and *Saccharomyces cerevisiae* to be safely included in rabbit diets. The Commission declared that potential harmful effects of most of the probiotic strains were tested in target animals. Application of multiple doses (100 to 10,000 fold) of probiotic microorganisms or spores had no negative influence on the weight gain or on the health status of animals. In this respect, Maertens and Ducatelle (1996) evaluate the tolerance of doe and weanling rabbits to 50 times (50 x 107 cells/g) overdose of live yeast, *Saccharomyces cerevisiae* Sc 47 (concentration 10¹⁰ CFU/g). The fattening trial confirmed that no
depressing effects on feed intake and weight gain. On the contrary, a tendency to increased performances was observed both before and after weaning when fed the supplement. Mortality did not occur in does, their offspring and the fatteners due to overdose of the probiotic. Histopathology did not reveal typical or more frequent lesions when the probiotic-overdose feed was fed to rabbits. Investigations of organs and tissues demonstrated no negative effect (European Commission, 2003). In the EU, *Bacillus cereus var. toyoi* (preparation of *Bacillus toyoi* var. toyoi having a minimum activity of \(1 \times 10^{10}\) UFC) is used in rabbits for fattening and for reproduction at levels in \(0.1 \times 10^9\) to \(5 \times 10^9\) CFU per kg of complete feeding stuffs and *Saccharomyces cerevisiae* (preparation of *Saccharomyces cerevisiae* having a minimum activity of \(5 \times 10^9\) UFC) is used in rabbits for fattening at levels in \(2,5 \times 10^9\) to \(5 \times 10^9\) CFU per kg of complete feeding stuffs (Anadón and Martínez-Larrañaga, 2000).

### PREBIOTICS

Prebiotics are a class of carbohydrates (oligosaccharides) that are not absorbed or digested in the small intestine of animals, but readily fermented by the intestinal microflora. This may result in changes in this flora, thereby increasing the number of beneficial micro-organisms, while repressing the harmful bacteria (Quigley, 2004).

Mannan/fructo/gluco-oligosaccharides are the most studied prebiotics in rabbit nutrition. Alltech (1994) presented the following comparison among fructo-oligosaccharides (FOS), mannan-oligosaccharides (Bio-MOS™) and gluco-oligosaccharides (MacroGrad):

<table>
<thead>
<tr>
<th>Monosaccharide unit (s)</th>
<th>FOS</th>
<th>Bio-MOS™</th>
<th>MacroGrad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>fructose</td>
<td>mannose/glucose</td>
<td>glucose</td>
</tr>
<tr>
<td>Raw ingredient</td>
<td>polymerization of fructose</td>
<td>yeast cell wall</td>
<td>yeast cell wall</td>
</tr>
<tr>
<td>Major role of action</td>
<td>pathogen cannot grow on it</td>
<td>pathogen cannot grow on it</td>
<td>may support growth of pathogens</td>
</tr>
<tr>
<td>Adsorption of pathogen</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Immune stimulation</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

Two hypotheses explain the action of commercial prebiotics in monogestrics; the first characterizes, only the mode of action of mannan-oligosaccharides probiotics, the phosphorylated mannan oligosaccharides, Bio-Mos®, derived from the outer cell wall of the yeast *Saccharomyces cerevisiae*, consists of a mannan component. The structure of the mannan resembles that of the carbohydrates on the animal gut wall. In theory, pathogenic, growth inhibiting microbes that normally adhere to mannans on the gut wall may instead bind to the mannan component of Bio-Mos®, where MOS have receptor properties for fimbriae of *E. coli* (sensitive to mannose) and *Salmonella spp.*, so these pathogens flushed out from the upper gut, and do not attach to the mucosal receptors. Elimination of the pathogens would presumably enhance the health and growth of the animal. While, the second, concerns the other sources of oligo-saccharides, where other types of oligosaccharides act as substrates for ‘desired’ microorganisms (Newman, 1994 and Huyghebaert, 2003).

**Mannan oligosaccharides (MOS):** Compared with the antibiotic, Zn-bacitracin (0.1 kg/ ton feed), Bio-Mos™ (1.0, 1.5 or 2.0 g/kg diet), they induced longer villi, increased absorption area and caecal VFAs, moreover, decreased caecal pH compared to the control not medicated (Pinheiro, et al., 2004b.). Also, providing Bio-Mos™ vs. oxytetracycline (20%), both at 0.2% at 28-46 day, then 0.1% at 47-70 day, equally, resulted in reduced mortality rate, improved feed conversion and live daily gain of rabbits (Fonseca et al., 2004). Radwan and Abdel-Khalek (2007) using Bio-Mos™ to manipulate the adverse impact of heat stress where the recorded rabbitry temperature exceeded 30°C, a supplemental level of 0.5% was effective to improve weight gain and feed conversion, without affecting caecal VFAs production or pH of rabbits. On the other hand, the study carried out by Ismail et al., (2004) showed that any of the studied Bio-Mos™ (0.5, 1.0 or 1.5 g/kg diet) did improve growth performance of rabbits. **Fructo-oligosaccharides (FOS):** FOS added at 0.24 % of rabbit feed improved significantly live weight gain, without holding effect on feed conversion, unexpectedly; the supplement increased the pH of
the caecal content (Aguilar et al., 1996). At 0.25%, FOS, partially reduced \( E. coli \) \( O103 \) and improved live growth weight, decreased caecal pH and \( \text{NH}_3 \) and increased VFAs production to levels not favorable to the presence of pathogenic \( E. coli \) (Morisse et al., 1992). Some other studies, indicated that FOS had no effect on growth performance (Lebas, 1996; at the rate of 0.34% during summer season) or curing against diarrhea or VFAs production when added to rabbit diet at the rate of 0.36 g/kg diet (Mourão et al., 2004).

**Gluco-oligosaccharides (GOS):** Results of two reported studies on GOS are controversial, on one hand, Gidenne (1995) found no effect on weight gain, and feed conversion and caecal VFAs pattern when rabbits fed on low fiber diets were offered GOS, more drastic, GOS increased morbidity and mortality rates. On the other hand, Radwan and Abdel-Khalek (2007) detected improved weight gain and feed conversion when summer heat stressed rabbits were fed on a balanced diet supplemented with GOS at the rate of 0.5%. The supplement had no effect on VFAs production or pH of the caecum.

**Galacto-oligosaccharides (GAS):** otherwise approved, the unique study on the effect of GAS on rabbit performance was reported by Peeters et al., (1992) who indicated that GAS at incremental level of 0.1 % up to 0.5% had no effect on live weight gain or feed conversion of rabbits, yet 0.1% partially inhibited the proliferation of \( E. coli \) and increased VFAs production.

**Safety and toxicity:**

No reported studies in rabbits, other livestock or human indicating that prebiotics incorporated in the diet at any studied level may induce bad consequences on animal. Up to our knowledge, prebiotics are just sugars that benefits the host good flora, and may be other factors rather than probiotics per se may affect the animal performance when a diet is supplemented with a probiotic, like that reported by Gidenne (1995) where he tried the interaction between GOS and dietary crude fiber level, the sharp decrease in the low crude fiber diet (9.2%) was more influential than providing GOS in the diet.

**REFERENCE:**


