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

A. M. Abdel-Khalek
Animal Production Research Institute, Dokki, Egypt.
aabdelkhalek_apri@hotmail.com

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 archea (22% and 12% at 28 and 70 days of age, respectively), protozoa and yeasts *(Saccharomyces guttulat*a 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 (Bacteroide*s) dominate the digestive flora in every segment of the intestine. Sporulating bacteria *(Clostridium, Endosporus* and *Acuformi*s), 100 to 1000 times less numerous than the *Bacteroide*s. 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 eminent 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 cerevisae* 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 (Ismail *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 in 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 NH_3 (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 cerevisae: Performance of growing rabbits were significantly improved under less favorable housing conditions, when fed diets supplemented with Biosaf® (a live yeast of *Saccharomyces cerevisae*) at the rate of 0.15 or 1.0% to provide 7.5×10^6 and 5×10^7 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 cerevisae* 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 cerevisae*; 2 g/ kg diet) or Lact-A-Bac; dried *Lactobacillus* *acidophilus* (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 cerevisae*, Sc 47; 10^6 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 10^9 *Bacillus cereus* var. *toyoi* spores/g) when added at the rate of 200 ppm (2 x 10^5 spores/g) or 1000 ppm (1 x 10^6 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 10^5, 1 x 10^6 and 5 x 10^6 *Bacillus toyoi* spores/g feed) caused a significant reduction and complete prevention of dirrahea 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 10^6 and 5 x 10^6 spores/g feed. spores of **Paciflor®** (Bacillus CIP 5832, 1.0 x 10^6 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 Gaàl, (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 NH_3 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 10^5 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 cerevisae* 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 10^{10} 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 x 10^{10} UFC) is used in rabbits for fattening and for reproduction at levels in 0.1 x 10^9 to 5 x 10^9 CFU per kg of complete feeding stuffs and *Sacharomyces cerevisae* (preparation of *Sacharomyces cerevisae* having a minimum activity of 5 x 10^9 UFC) is used in rabbits for fattening at levels in 2,5 x 10^9 to 5 x 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-oligosaacharides are the most studied prebiotics in rabbit nutrition. Alltech (1994) presented the following comparison among fructo-oligosaacharides (FOS), mannan-oligosaacharides (Bio-MOS™) and gluco-oligosaacharides (MacroGrad):

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

Two hypotheses explain the action of commercial prebiotics in monogestrics; the first characterizes, only the mode of action of mannan-oligo-saccharides probiotics, the phosphorylated mannan oligosaccharides, Bio-Mos®, derived from the outer cell wall of the yeast *Saccharomyces serivisae*, 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 oligosaacharides (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℃, 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-oligosaacharides (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 caceal content (Aguilar *et al.,* 1996). At 0.25%, FOS, partially reduced *E.coli O103* and improved live growth weight, decreased caecal pH and 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-oligosaacharides (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-oligosaacharides (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 sugers 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.

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