Possible alternatives to the use of antibiotics as growth promotors. New additives

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SUMMARY - The non-therapeutic use of antibiotics may be reduced by administering both microbial cultures and molecules such as oligosaccharides and lectins. In the former case, an attempt is made at preventing intestinal pathogens from settling down, by administering microorganisms which can colonize the digestive tract and leave out all dangerous bacteria. The microorganisms mostly used in connection with monogastric animals are bacteria from the *Bacillus, Bifidobacterium* and *Lactobacillus* genera and yeasts from *Saccharomyces cerevisiae* genus. The use of *S. cerevisiae* and *Aspergillus oryzae* is popular among adult ruminants, leading to weight gain both in calves and bullocks. In pre-ruminant cattle, the use of lactic bacteria (*Lactobacillus* and *Bifidobacterium*) also deserves some interest. In the field of chemical probiosis, the use of fructo- and gluco-oligosaccharides, capable of selectively stimulating the lactic bacteria, has yielded very interesting results both in monogastrics and in calves. Equally promising is the dietary supplementation with oligomannans and lectins. Such compounds saturate and bind to the enterocyte receptors which are present on the cell walls of pathogenic bacteria, thus preventing them from colonizing the intestinal lumen.

Key words: Antibiotics, probiotics, additives.

***RESUME -** "Alternatives possibles à l'utilisation d'antibiotiques comme promoteurs de croissance. Nouveaux additifs". L'utilisation non thérapeutique des antibiotiques peut être réduite soit par des molécules comme les lectines et les oligosaccharides soit par l'administration de cultures microbiennes. Dans le premièr cas on cherche à empêcher l'installation des pathogènes intestinaux en administrant des micro-organismes qui colonisent l'appareil digestif en excluant les germes dangereux. Les micro-organismes les plus utilisés chez les monogastriques appartiennent aux genres* Bacillus, Bifidobacterium *et* Lactobacillus *parmi les bactéries et aux* Saccharomyces cerevisiae *parmí les levures. Parmi les ruminánts adultes est diffusé l'emploi de* S. cerevisiae *et de* Aspergillus oryzae, *qui ont permis les augmentations de poids soit chez les bouvillons soit chez les veaux. Chez le bovin pré-ruminant il faut mentionner l'emploi des bactéries lactiques* (Lactobacillus *et* Bifidobacterium). *Dans le domaine de la probiotique chimique l'utilisation de fructo et gluco oligosaccharides qui peuvent stimuler sélectivement les bactéries lactiques, a permis d'obtenir des résultats très intéressants chez les monogastriques et dans les veaux. L'intégration alimentaire avec des oligomannanes et lectines est également prometteuse. Les oligomannanes et les lectines sont des composés qui se lient, en les saturant, aux récepteurs pour les entérocytes présents sur les parois cellulaires des bactéries pathogènes en les empêchant de coloniser la lumière intestinale.*

***Mots-clés :** Antibiotiques, probiotiques, addititifs.*

Introduction

Adequate control of the activity of microorganisms living in symbiosis with the higher animal in the digestive tract of ruminants and/or monogastrics is essential to ensure balanced biological activity, ability to react to stress under intensive farming conditions, good immune response, adequate health status, excellent reproductive performance and reduced environmental impact.

Since the early 50's, thanks to the use of antibiotics as additives promoting performance in animal nutrition, considerable improvements have been obtained in production.

The non-therapeutic use of antibiotics has the following aims:

Prophylactic purposes: (i) in young animals, to reduce the enteric diseases whose onset is favoured by incomplete development of the immune system during the first weeks of life; and (ii) in adult animals, to prevent the onset of feed-induced pathologies (acidosis in steers).

Animal breeding purposes: The lower degree of microbial contamination in the gut causes less development in the intestinal lumen (Gaskins, 1996), with reduced energy and protein requirements for re-synthesizing the enteric cells and greater availability of nutrients to be used for production purposes. In oloxenic animals the enterocyte turnover is accelerated by approximately twice versus the axenic animals (Vanbelle *et al.*, 1990).

Antimicrobials have also proved to be important for sustainable livestock production and for the control of animal infections that could be passed on to humans. However, concerns about using antibiotics in livestock is growing. In 1995, the WHO set up a special working group to assess this problem. More recently, the treatment of enteric diseases in children living in developing countries was found to be increasingly difficult, due to the spreading of resistance to antibiotics among the human intestinal pathogens.

"The magnitude of the medical and public health impact of antimicrobial use in food animal production is not known. It is unrefuted that the use of antimicrobials leads to the selection of resistant bacteria and that the scope of the emerging problem depends, among other things, on duration of exposure to and concentration of the antimicrobial. Residues of antimicrobial agents in food of animal origin in excess of the agreed acceptable minimum residue levels (MRLs) may contribute to generation of resistance in bacteria in humans. However the current evidence suggests that the risk is low. Of more concern may be that such residues could indicate inappropriate use of antimicrobials by producer. The medical consequences of resistance acquisition in bacteria of animal origin are highlighted by the following examples.

Salmonella-Campylobacter - Following the introduction of fluoroquinones for use in food-producing animals the emergence of *Salmonella* serotypes and *Campylobacter jejuni* with reduced susceptibility to fluoquinones in humans has become a cause of particular concern.

Enterococci - The increase of glycopeptide-resistant enterococci from animals, linked to the use of some glycopeptides grow promotor (e.g., avoparcine) can reach humans via the food chain. Glycopeptide-resistant enterococci cause serious infections in hospitalized immune-impaired patients. There is concern that there will be increased dissemination of glycopeptide resistance genes to *Enterococcus faecalis* and their spread to other gram-positive organisms, particularly to multiresistant *Staphylococcus aureus* for which vancomycin is the drug last resort. This medical impact would be greatest in countries where vancomycin is used intensively; in the United States of America for instance vancomycin is used intensively and avoparcine has never been used.

Escherichia coli - Multiresistant *E. coli* have been selected by the use of abroad spectrum antimicrobials in both livestock and humans. The development of antimicrobial resistance in *E. coli* creates problems due to their high propensity to disseminate antimicrobial resistance genes. Resistance genes have been traced from *E. coli* in animals to *E. coli* in humans. Certain *E. coli* are foodborne pathogens.

Because of the growing global need for food and potential public health consequences of the transmission of resistant bacteria through the food chain, the objectives for risk management at the animal production level are to assure the efficient production of safe and wholesome food of animal origin for human consumption and to reduce potential public health risks associated with farming practices to enable the growth of the global food supply" (from WHO meeting Berlin, Germany, 13-17 October 1997). Recently, (February 2, 1988) the Swedish Government requested a ban for the non therapeutic use of antibiotics.

However, one cannot possibly think of imposing a sudden and generalized ban on such products since this would imply severe repercussions on the food supplies to the poorest countries. The FAO has in fact estimated that, should antibiotics be banned from animal breeding as performance promoters, one would be faced with a 30% reduction in the availability of proteins from animal origin throughout the World, which would certainly make the state of chronic malnutrition in developing countries even worse.

With the appearance of the first problems related to the use of antibiotics, the possibility of using probiotics as growth promoters or with prophylactic effects began to be seriously considered for scientific purposes. Probiotics are "*live microorganisms capable of inducing a beneficial effect on the balance of microorganisms in the digestive tract*".

With Directive 93/113, the EU acknowledged the validity of this category of products as "additives", in that they improve the production performance and the quality of products of animal origin.

One of the most important advantages of such additives is the absence of undesired antibiotics residues in meat, milk and eggs.

A first tentative list of products authorized in the different EU countries is reported in the Official Journal of the European Community of September 11, 1996 - C 263. Apart from positive results, the use of microorganisms has at times yielded a disappointing response. The method of administration has in fact very much affected the results. A critical point was, above all, the possibility of using a substrate (feedstuff) capable of favouring the development of useful microorganisms and affecting the adhesion of undesired or pathogenic microorganisms to the gut walls. It is non only a matter of mechanically controlling the competition between microorganisms, shifting it towards those that are regarded as useful. It is rather a question of acting on the metabolism of microorganisms and of the gut wall, in order to control the metabolic activity of the digestive tract as a whole. It is therefore necessary to act nutritionally on the digestive tract microorganisms by way of increasing the useful microorganisms, perhaps via their exogenous supply, at the same time providing the compounds which may favour competition with the undesired microorganisms and positively control metabolism.

Delzenne and Roberfroid (1994) provided the scientific background for the characterization of such feedstuffs as pre-biotics in the sense of "*undigestable dietary ingredients which positively affect the host by beneficially and selectively stimulating the growth and/or activity of a limited number of bacteria*".

Pre-biotics act by stimulating the microorganisms which are present in the intestinal tract and not by integrating them, as is the case for probiotics. Pre- and probiotics do not exclude each other's function and can, or better, must be used simultaneously in order to obtain a powerful synergistic effect.

The concept of competitive exclusion and the intestinal microflora

From a microbiological point of view the concept of competitive exclusion implies the prevention of establishment of one microorganism into an environment because that habitat is already occupied by a competing microorganism better suited to maintain itself in that environment. The gut and the intestinal microflora represent a complex ecosystem in which several factors affect the composition of the microbial flora. Spring (1996) listed the regulatory mechanism involved in the regulation of the microbial ecology of the gut (Table 1).

A good knowledge of the composition of the GI microbial ecosystem and its control mechanism is required in order to improve the effectiveness of using commercial microbial probiotic cultures in animal production.

Chemical probiosis

Several molecules may play a pre-biotic role, promoting the development of certain microbial groups; microorganisms, in fact, do not use the energy and protein sources in the same fashion and have different needs for micronutrients and vitamins. It is therefore possible to stimulate the growth of special microbial species through the supply of certain substrates.

In ruminants, interesting results were obtained, both *in vivo* and *in vitro*, with the use of amino acids and organic acids (Masoero *et al.*, 1992, 1995) or peptides (Chen *et al.*, 1987). As the main category of probiotics, oligosaccharides have attracted a great deal of commercial attention; several types are currently produced and used as "additives" for breeding animals: fructo-oligosaccharides

(FOS), gluco-oligosaccharides (GOS), mannano-oligosaccharides (MOS), galacto-oligosaccharides (GAS), xilo-oligosaccharides (XOS). They may derive from plant origin (FOS and galacto-oligosaccharides), from enzymatic polysaccharide hydrolysis (FOS and XOS) or be re-synthesized *de novo* (FOS, GOS, GAS). Their limited inclusion in the diet (usually 0.1-0.3%) may improve weight gain, the feed conversion ratio and the health status. The size of such effects, however, is affected by numerous factors such as the type of "additive", the age of the animals, the species and the breeding conditions. In the field of monogastric nutrition, the use of fructo-oligosaccharides (FOS) gave good results in rabbits (Morisse *et al.*, 1992, 1993).

Table 1. Autogenic regulatory mechanism which affect the composition of the intestinal microbial flora (From Spring, 1996)

Regulatory mechanism	Control factors
Nutrient utilization	Competition for nutrient or growth factors Synergistic nutrient utilization
Attachment	Competition for receptor sites Stimulation of enteric cell turnover
Creation of a restrictive environment	pH Lactic acid production VFA production H_2S Eh Resistance to bile salts Induction of immunologic process
Productions of antimicrobial substances	NH_3 H_2O_2 Hemolysin Bacterial enzymes Bacteriophage Bacteriocins Antibiotics

The mechanism of action of such products most likely implies selective stimulation of special positive microbial clusters in the gut, such as *Bifidobacterium* (Unno *et al.*, 1993; Hirayama *et al.*, 1994; Howard *et al.*, 1995), *Bacteroides* and *Lactobacillus* (Takahashi et *al.*, 1996), *Pediococcus* spp. o *Enterococcus faecium*, but not *Salmonella typhimurium* (Oyazarbal and Corrier, 1996). Differences may, however, be found between strains of different species of the *Bifidobacterium* genus. The microorganisms of animal origin use inulin (a FOS) more efficiently than those isolated from the human gut. The same strains, however, are not capable of deriving energy from levans; it was also pointed out that the FOS which are best metabolized are those containing up to 5 fructose residues (McKellar *et al.*, 1993).

The efficacy of dietary integration with oligosaccharides is also modulated by the type of diet, being greater in hamsters fed with a diet containing a large amount of bran versus those receiving a feedstuff with greater meat proportion (Hirayama *et al.*, 1994). It is not only the type of diet which affects the response to FOS administration; 3 different strains of *Bifidobacterium* (*B. infantis* ATCC 15697, *B. adolescentis* ATCC 15703, *B. longum* ATCC 15707), showed different growth patterns in culture mediums added with 3 different types of fructane, a natural (extracted from *Helianthus tuberosus*) and two commercial ones (Yamazaki and Matsumoto, 1994). Galacto-oligosaccharides, even if to a lower extent that FOS, are another substrate which is capable of promoting the growth of *Bifidobacterium* and *Lactobacillus* (Morishita *et al.*, 1992). By administering GOS (polymerization grade from 1 to 7, with prevalence of units with 5 residues) to axenic rats, Valette *et al.* (1993) established that such molecules are resistant to intestinal digestion; if the same animals are inoculated with human intestinal microflora, the subsequent administration of GOS does not change caecal pH, VFA production and lactic acid concentration. Changes are in fact observed in terms of the VFA profile (reduced molar percentages of butyric, isobutyric, isovalerianic acids and increased molar percentage of caproic acid) with increased production of H_2 and CH_4. Reduced production of branched chain VFA may point to a decrease in the proteolytic activity of the large intestine.

The administration of FOS increases the intestinal absorption of Ca, Mg, P (Ohta *et al.*, 1994; Ohta *et al.*, 1995a; Baba *et al.*, 1996) and Fe (Ohta *et al.*, 1995b) in rats, perhaps due to the effect of enzymatic hydrolysis performed by Lactobacilli on compounds which chelate such minerals, or by virtue of a lowering of the pH induced in the colon. The results obtained by Ohta *et al.* (1994) show that the absorption sites and mechanisms may considerably differ for these minerals.

FOS, galacto-oligosaccharides, malto-oligosaccharides (MO) and raffinose (RF) differ in their ability to stimulate mineral absorption; FOS are those that yield the best results, RF and galacto-oligosaccharides have an intermediate effect, while MO have no effect (Ohta *et al.*, 1993). Reduced lipidemia and liver lipid deposition have been observed in experimental animals following the inclusion of FOS in the diet (Otsuka and Kubo, 1995).

Positive results on reduction of pathogens intestinal colonization by feeding animals with MOS is probably due to the capacity of these sugars to bind to pathogenic organisms such as *Salmonella typhimurium* (Oyofo *et al.*, 1989) and *Escherichia coli* or stimulate the immune system. Both these mechanisms have a common feature: both the cell receptors and the antigenic determinants of several pathogenic bacteria contain mannans (Castro *et al.*, 1994; de Ruiter *et al.*, 1994; Kagaya *et al.*, 1996). Some oligomannans are deliberately included in vaccines as adjuvants which may enhance and prolong an immune response. If administered through the diet, oligomannans would compete with the corresponding intestinal receptors, which are substrates for the adhesion of the gut pathogens, thus reducing their ability to form colonies in the digestive tract epithelia. The same approach based on competitive adhesion between bacterial adhesion sites, enteric cellular receptors and molecules can be also use for lectins. A snowdrop-extracted lectin (GNA) showed, apart from high specificity for oligomannans, the ability to stop the growth of *E. coli* type I in the gut of rats (Pusztai *et al.*, 1993). This strategy may also be extended to the ability, by some sugars, to bind to bacterial toxins (Stoll *et al.*, 1980). A risk linked to the addition of this substance is represented by the possible increase in intestinal colonization by *S. typhimurium* due to the formation of bridge bindings between the bacterial cells and the enteric mucosa (Abud *et al.*, 1989; Pusztai *et al.*, 1990).

The administration of oligomannans to rats can stimulate macrophage activity (Newman, 1995); this probably accounts for the lower incidence of lung diseases found in calves receiving commercially available oligomannans (Newman *et al.*, 1993). Furthermore, their ability to stimulate the enteric receptors recognized by pathogenic bacteria allows them to bind to such bacteria, thus reducing their availability in the digestive tract (Spring *et al.*, 1996).

No information is available as to the effects that such products have on the processes which take place in the large intestine of ruminants. It is in fact most likely that such products cannot perform their action in this portion of the digestive tract, since they possibly undergo complete breakdown in the rumen. It is, among others, quite likely that a lack of fermentation energy systematically occurs in the large intestine. For this reason, the control of blood urea necessarily implies monitoring the energy availability in this part of the digestive tract.

New additives for pigs

Microbial cultures

Bacteria

An assessment of the results which can be obtained through the use of microbial cultures is made more complex by the at times contemporary use of antibiotics which adds to other variability factors such as: (i) age of the animal; (ii) diet; (iii) probiotic dose; (iv) microbial genus and species; and (v) viability and specificity of the microorganism.

An improvement in the health status is generally found together with reduced mortality, less consistent are, however, the effects on weight gain and feed conversion (Vanbelle *et al.*, 1990). The size of response is also linked to inadequate gastric production of HCl in piglets, which favours the settlement of pathogens in the gut. Similarly to acidifiers, the administration of lactic bacteria causes a drop in pH which may offset the lower acid secretion in the abomasum. It is not by chance that the best results are obtained with piglets early after weaning fed with a diet of plant origin containing no milk.

The results obtained during weaning are quite inconsistent, even if pointing to a certain efficiency for the addition of lactic bacteria (*Lactobacillus* and *Bifidobacterium*), alone or in combination with strains of *Streptococcus faecium*. Apart from the above factors which may affect efficiency, the following should also be mentioned:

(i) Use of different species from the *Lactobacillus* genus (*L. casei*, *L. bulgaricus*, *L. acidophilus*).

(ii) The fact that not all *Lactobacili* isolated from the animal gut are capable of effectively resisting the pathogens. Hillman and Fox (1994) pointed out that only 3 out of 31 strains of *Lactobacillus*, isolated from pig stool, are in fact capable of strongly inhibiting the growth of *Escherichia coli* O149.

Apart from the ability to colonize the intestinal epithelium by adhering to it and producing lactic acid or bacteriocins, part of the probiotic effect of lactic bacteria could be due to the power of aggregation against *E. coli* shown by strains of *L. acidophilus* and *L. salivarius* (Spencer and Chesson, 1994).

The addition of *Bacillus subtilis* spores increases the number of lactobacilli while decreasing the number of coliform bacteria (Bonomi *et al.*, 1995; Kornegay and Risler, 1996; Maruta *et al.*, 1996). This effect, however, was found to be linked to the type of *B. subtilis* strain which is used (Kornegay and Risler, 1996). Maruta *et al.* (1996) also found a drop in the populations of Clostridia, Streptococci and Enterobacteriaceae. However, the changes induced in the intestinal microbial population have not been such as to significantly affect production performance (Martelli, 1992; Kornegay and Risler, 1996). Also less recent works had found less than 5% improvement in the daily weight gain (Peo, 1984; Trotters, 1984) or even negative variations (Pollman *et al.*, 1984).

By adding *Bacillus toyoi* spore to sow (300 ppm) and piglet (20 ppm) diets Gunther (1994) improved the weaned litter size (+0.55 piglet/sow), the growth (5.8-8.1%) and feed (3.72-6.20%) efficiencies.

Yeasts

Even if some studies (Bertin and Tournut, 1994; Roques *et al.*, 1994; Kornegay *et al.*, 1995) found better performance following the use of yeasts, only one case showed a positive effect on the microbial composition of the gut, with reduced number of pathogens (Roques *et al.*, 1994). In other studies (Jost *et al.*, 1993; Veum *et al.*, 1995; Bekaert *et al.*, 1996) no improvement in breeding performance was found in association with the administration of cultures of *Saccharomyces cerevisiae*. Even if showing no improvement in the digestibility of fibre fractions, Kornegay *et al.* (1995), were able to obtain an increase in the daily weight gain only in high-fiber diets containing 8% of peanut hulls. This would seem to indicate greater metabolic activity on the part of the cellulosolytic microflora of the pig caecum, resulting in greater availability of volatile fatty acids to the animal.

The better growth and feed conversion obtained by Savoini *et al.* (1996) using cells of *S. cerevisiae* enriched with Cr are also due to the growth promoting action of this mineral and not only to the effect of the yeast on the intestinal microflora.

Feed induced anti secretory proteins (ASP)

Intestinal fluid secretion induced by enterotoxins may be inhibited by anti secretory proteins (ASP) (Lange and Lönnroth, 1984). These regulatory protein are synthesized in the central nervous system

(Lönnroth and Lange, 1986) and transported via the blood and bile to gut; they seem to play an important role against enteric disease (Gorannson *et al.*, 1993). Sow's milk contains ASP and suckling pigs absorb it from the intestine by passive absorption (Sigfridsson *et al.*, 1995), ASP blood level decreases after weaning (Lange *et al.*, 1993) exposing piglets to enteric diseases. Supplementing creep feed with sugars, sugar alcohols and pure aminoacids can increase the production of feed induced lectines (FIL) which have the same antisecretory effects as ASP but a slightly different chemical structure (Lönnroth and Lange, 1986). Table 2 shows the results of field experiments comparing the effect of control and ASP-inducing diet on animal health and performances.

Table 2. The influence of a FIL-inducing diet in the production performance in 3 farms (modified from Goransson *et al.*, 1993)

Farm	No. of pigs	Daily weight gain (g) 0-35 d post weaning		ASP (units/ml plasma) 4 d post weaning		Post weaning diarrohea (%)	
		C	FIL	C	FIL	C	FIL
1	40	202	247	0.42	0.87	35	10
2	54	266	325	0.79	1.05	15	4
3	325	284	380	0.76	0.94	31	2
Differences (%)			+26.6		+43.94		-80.26

The experimental diet caused an increase in ASP blood level and a reduction in clinical post weaning diarrhoea, with higher daily weight gain versus the control diet. The ASP plasma level sharply decreased in the first week after weaning, probably due to stress. On the contrary the blood FIL concentration did not fall, thus providing better protection against diarrhoea in young piglets.

Acidifyers

Supplementing the diets of weanling pigs with organic acids, such as citrate, formate, fumarate or propionate gives inconsistent responses in terms of performances. As reported by Ravindran and Kornegay (1993) citrate supplementation to the weaner piglet diets modified from -11.3 to 14.3% and from -6 to 11.1%, compared to control, the daily gain and feed conversion efficiency, respectively. The best responses were usually obtained during the first 3 weeks of age probably because of insufficient gastric production of HCl and with level of acid supplementation higher 30 g/kg of feed. Generally, improvements in feed efficiency tended to be more consistent than body weight gain improvements.

Several studies have reported consistent improvements in weight gain and feed conversion with the addition of fumaric acid to weaner diets, but other experiments have not provided any evidence of positive effects of fumarate supplementation on animal performances (Easter, 1988; Ravindran and Kornegay, 1993). Based on what these Authors have reported, also fumaric acid shows a dose-related effect similar to that of citrate, with increased efficacy above 20 g/kg supplementation. Also in this case the parameter mostly affected by treatment is feed conversion (Table 3).

Bolduan *et al.* (1988), Sweet *et al.* (1990) and Mathew *et al.* (1991) reported improved growth rates when weaner diets were supplemented with propionic acid (from 0.1 to 1%). In contrast, Giesting and Easter (1985) and Thacker *et al.* (1992) reported that the addition of 2 or 2.5% of propionate had no beneficial effect on the growth or feed conversion of weaner pigs; Giesting and Easter (1985) also found depressed feed intake possibly due to the pungent aroma of propionic acid.

Eckel *et al.* (1992) fed early-weaned pigs with 6, 12 and 18 g/kg of formic acid and obtained improved post-weaning growth (+23, 31 and 29% respectively); similar results were obtained by Eidelsburger *et al.* (1992a). A depression in feed intake was recorded with supplementation levels higher than 18 g/kg. The administration of formic acid reduces NH_3 concentration in the large intestine (Eidelsburger *et al.*, 1992a; Gedek *et al.*, 1992; Roth *et al.*, 1992b) and changes the composition of the intestinal microflora with increased availability of coliform bacteria (Gedek *et al.*, 1992a; Kirchgessner *et al.*, 1992a,b), reduction in *Bacteroides* (Gedek *et al.*, 1992a; Kirchgessner *et al.*, 1992a,b) lactic bacteria (Gedek *et al.*, 1992a) or lactic acid concentration; in contrast, increased production of acetic acid was also found (Eidelsburger *et al.*, 1992a; Roth *et al.*, 1992a).

Table 3. Summary of published data on the effects (% compared to control) of organic acids supplementation on the performance of weaner pigs (modified from Ravindran and Kornegay, 1993)

Treatments		No. of trials	Daily gain	Feed intake	Gain/feed
Citric acid (g/kg)	10	5	+2.88	+4.28	+0.66
	15	5	-0.66	-2.82	+1.58
	20	4	+1.42	-3.10	+6.95
	30	4	+6.17	+7.37	+0.4
Citric acid (age of pigs, d)	7-10	4	+4.48	+9.30	-0.53
	20-21	7	+5.01	+4.54	-0.19
	25-32	9	-0.43	-4.05	+4.81
Fumaric acid (g/kg)	10	2	+2.95	-2.10	+1.15
	15	5	-0.62	+0.96	-0.16
	20	4	+5.93	-1.70	+7.50
	30	4	+4.05	-2.20	+5.88

Numerous hypothesis have been put forward concerning the mode of action of acidifiers, all linked to the effects induced by reduced gastric pH, such as increased nutrient digestibility (Eckel *et al.*, 1992; Eidelsburger *et al.*, 1992b; Kirchgessner *et al.*, 1992a,b; Thacker *et al.*, 1992) following more effective proteolytic enzyme activation. In contrast, other Authors (Bolduan *et al.*, 1988; Giesting and Easter, 1991; Mosenthin *et al.*, 1992; Gabert and Sauer, 1995) have not found any improvement in nutrient utilization.

The use of organic acid can reduce *E. coli* colonization in the gut (Bolduan *et al.*, 1988; Mathew *et al.*, 1991; Eckel *et al.*, 1992; Gedek *et al.*, 1992b, Isobe *et al.*, 1994) as well as the incidence of diarrhoea (Eidelsburger *et al.*, 1992b); other works have not pointed to any effects on enteric disorders or on a reduction in the number of intestinal coliform bacteria (Sutton *et al.*, 1991; Gedek *et al.*, 1992a; Kirchgessner *et al.*, 1992a,b; Risley *et al.*, 1992, 1993).

The intermediate organic acids of Kreb's cycle might act as energy sources inducing more efficient energy metabolism (Kirchgessner and Roth, 1982). Better efficiency of acidifying treatments could be obtained by resorting to their joint administration with probiotic bacteria (Johnson, 1992) while encapsulation with a protective matrix (Maxwell *et al.*, 1993) might turn out to be useful in adult subjects where the problem is not so much an insufficient gastric secretion of HCl but rather an adequate control of caecal fermentations.

According to Galfi and Neogràdys (1996) Na-salts of *n*-butyrate, if added in a percentage of 0.17% (on DM basis) to the diet, reduce intestinal colonization by pathogenic *E. coli* and increase the number of Lactobacilli. The proposed mode of action of *n*-butyrate is related to an ionophore-like action of this acid and not to a pH-lowering effect.

Essential oils

Some higher vegetable species (anise, origan, rosemary, pepper, celery, thyme, etc.) contain essential oils which give them aromatic properties. If added to the diet of fattening pigs, such substances considerably improve their growth pattern (Table 4).

Table 4. Performance of fattening pigs fed control or supplemented (flavours, antibiotics) diets (modified from Gunther, 1991)

Items	Negative control	Flavoured diets		Salocin control
		75 g/t	112.5 g/t	20 mg/kg
Weight gain 70 d (kg)	69.60[a]	73.20[b]	75.75[b]	75.40[b]
Weight gain 70-120 d (kg)	35.55[a]	37.65[b]	39.20[c]	37.85[bc]
Daily weight gain (g)	628.3[a]	685.4[b]	709.6[c]	704.6[bc]
Relative weight gain (%)	100	109.1	112.9	112.1
Feed conversion ratio	3.11	2.93	2.95	2.95

a,b,c: $P<0.01$

Gunther suggests that such effects are due to: (i) lower amino acid oxidation; (ii) an antibiotic-like action against the intestinal microorganisms, with reduced thickness of the villi and, consequently, reduced protein metabolism of the enterocyte; (iii) increased activity of the digestive tract enzymes; and (iv) greater food intake because of improved palatability of the feedstuff.

Experimental evidence is currently available only for bactericidal action (Brud and Gora, 1990) and dry matter intake (Baidoo *et al.*, 1986; Villalba and Provenza, 1996), while the results concerning increased activity of the digestive tract enzymes are more uncertain (Cerioli and Fiorentini, pers. comm.).

New additives for poultry

The integration of FOS into diets fed to poultry inoculated with *S. typhimurium* versus controls causes a reduction in the intestinal colonization by this pathogen and also an improvement in the daily weight gain and feed conversion ratio (Choi *et al.*, 1994; Oyazarbal and Corrier, 1996). On the contrary, no effects were found on the dressing percentage and the meat fat content (Waldroup *et al.*, 1993). The contemporary use of FOS and cultures of probiotic bacteria makes it possible to establish interesting synergies in the fight against salmonellosis (Table 5). Several trials have shown a considerable reduction in the number of intestinal pathogens following the administration of mixed microbial cultures from faecal suspensions of healthy subjects both experimentally (Hinton *et al.*, 1991; Blankenship *et al.*, 1993; Kogut *et al.*, 1994; Hakkinen and Schneitz, 1996; Hume *et al.*, 1996) and in field conditions (Wierup *et al.*, 1992). Recording of such microbial populations by the health authorities implies that they have been microbiologically characterized (Stavric, 1992). This requires the identification of less complex mixtures which can compete with the pathogens. Schoeni and Wong (1994) were able to obtain markedly reduced intestinal colonization by *Campilobacter jejuni* with the use of mixtures containing *Citrobacter diversus*, *Klebsiella pneumonie* and *E. coli.*

The intestinal *S. dublin* and *E. coli* concentrations were reduced when oligomannans were added to broiler chicks diets (Spring *et al.*, 1996) (Fig. 1). The most probable mechanism involved is the saturation of bacterial intestinal-wall receptors reducing the bacterial chance of colonizing the intestine.

The contemporary administration of probiotic microorganisms and sugars (lactose, mannose, FOS) improves the antagonist action against *C. jejuni* (Table 6).

Table 5. Effect of 0.75% FOS in the feed and administration of an undefined bacterial culture (BC) on *Salmonella* colonization of 7-day-old chicks (Bailey *et al.*, 1991)

Treatment	Challenge level	% *Salmonella* positive chicks
None	10^6	47.5
FOS	10^6	36.4
BC	10^6	43.5
FOS + BC	10^6	10.5
None	10^9	.95.5
FOS	10^9	87.0
BC	10^9	60.9
FOS + BC	10^9	19.0

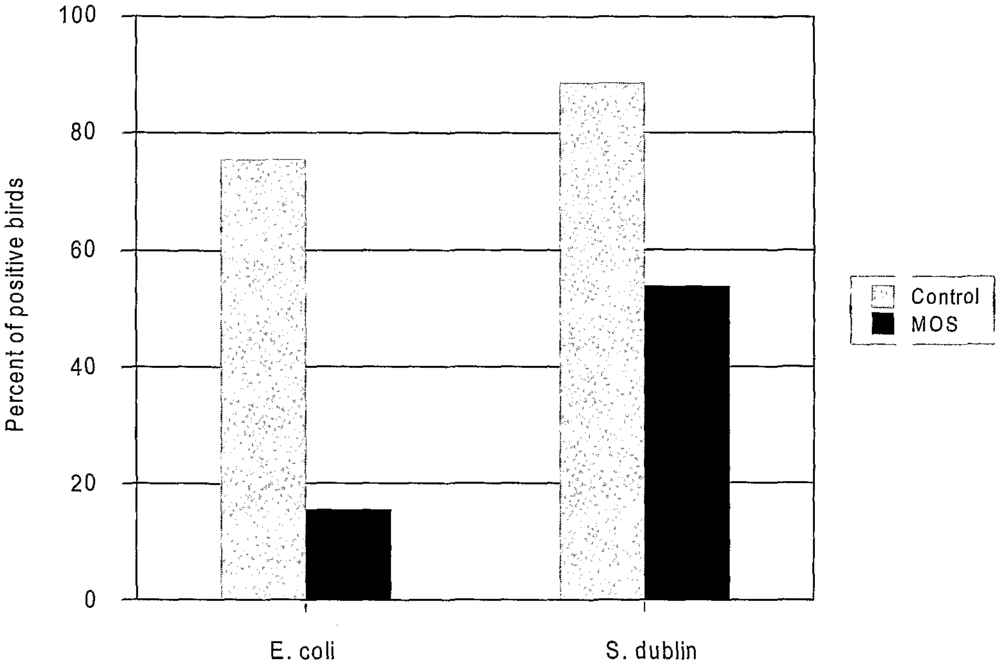

Fig. 1. Effects of oligomannans addition in broiler chicks diets on intestinal *E. coli* and *S. dublin* concentrations.

Similarly, other Authors found an improvement in the reduction of intestinal colonization by *Salmonella* by administering lactose (5-10%) to animals who had already received CE cultures (Corrier *et al.,* 1991; Ziprin and Deloach, 1993; Kogut *et al.,* 1994). Also some strains of *Lactobacillus* spp. were found to reduce the number of coliform bacteria in the caecum (Jin *et al.,* 1996a; Rada and Marounek, 1997) and of *Salmonella* (Jin *et al.,* 1996b); the presence of lactose in the diet enhances this protective action (Quinn *et al.,* 1995). An interesting side-effect found in conjunction with the administration of *Lactobacillus* is a reduction in the cholesterol content of the egg yolk (Haddadin *et al.,* 1996; Mohan *et al.,* 1996), due to the production of a carbohydrate which binds to cholesterol and either prevents its intestinal resorption or causes its precipitation in an insoluble form increasing its elimination through the faeces rather than its resorption by the gut. Better performances (body weight gain, feed/gain) were obtained also following the administration of *S. faecium* (Owings *et al.,* 1990) *Lactobacillus* spp. plus *B. bifidum*, plus *A. oryzae* and *Torulopsis* (Mohan *et al.,* 1996), *L. reuteri*

(England *et al.,* 1996) *Lactobacillus* spp. and *B. subtilis* (Jin *et al.,* 1996) *L. acidophilus* plus *B. subtilis* plus *S. faecium* (Chiang and Hsieh, 1995). The use of acidifiers significantly reduces intestinal colonization by *Salmonella* only if in combination with microbial cultures (Hinton *et al.,* 1991) but enhances growth in broilers (Skinner *et al.,* 1991).

Table 6. Ability of defined microbial cultures† (MC) and carbohydrate treatments to prevent intestinal colonization by *C. jejuni* (Modified from Schoeni and Wong, 1994)

Treatments	No. of trials	No. of chicks	% of positive chicks	Cecal pH
Control	6	37	61.6	5.7
MC	5	27	20.2*	5.8
Lactose with:				
No. MC	4	25	31.1	5.1*
MC	4	27	40.8	5.5*
Mannose with:				
No. MC	4	25	12.9*	5.7
MC	3	25	0.0*	5.8
FOS with:				
No. MC	4	25	7.7*	5.4*
MC	4	25	14.9*	5.3*

†Citrobacter diversus, Klebsiella pneumonie and *E. coli*
*Significantly different (P<0.05) from the control value

Essential oils

Similarly to Gunther (1991), also Piva *et al.* (1991) found that dietary supplementation with essential oils improves the performance of broilers also in comparison with diets supplemented with virginiamycin (Table 7).

Table 7. Body weight gain of broilers fed diet with or without additives (virginiamycin or essential oils) (From Piva *et al.,* 1991 modified)

Average weight (g/head)	With virginiamycin		Without virginiamycin	
	Control	Treated†	Control	Treated†
1-29 days	964.57^{a}	968.72^{a}	965.75^{a}	991.06^{b}
29-44 days	1867.81^{ABb}	1981.74^{Bc}	1779.09^{Aa}	1932.39^{Bbc}
44-61 days	2944.09^{ABb}	3022.50^{Bbc}	2839.56^{Aa}	3064.35^{Bc}

†with CRINAROM 737
a,b,c: P<0.05; A,B: P<0.01

New additives for rabbits

In rabbits, the administration of FOS reduces the pH and the NH_3 concentration in the caecum, increases the production of VFA and favours the presence of non-pathogenic strains of *E. coli* (Morisse *et al.*, 1993) (Table 8).

Table 8. Effect of adding fructo-oligosaccharydes on rabbit health status, performance and caecum content (Modified from Morisse *et al.*, 1993)

Treatments	pH	*E. coli* (log $_{10}$/g)	Enteritis (%)	NH_3 (mM/kg)	VFA (mM/kg)		
					C_2	C_3	C_4
Control	6.26^b	2.5^A	46.4^B	17.0^B	45.7^a	1.3	9.2^a
Treated	6.04^a	4.2^B	14.8^A	11.1^A	57.6^b	1.8	19.8^b

a,b,c: $P<0.05$; A,B: $P<0.01$

Luicke *et al.* (1992) could not find any differences in weight gain, in the feed conversion ratio or in the composition of the faecal contents of rabbits who had previously received FOS. Dietary supplementation with microbial cultures may be considered as an alternative to the use of antibiotics.

Contemporary administration of *L. acidophilus, Streptococcus faecium* and *S. cerevisiae* (LACTO-SACC) increases the digestibility of the diet (Gippert *et al.*, 1992; Yamani *et al.*, 1992; Kamra *et al.*, 1996), reduces the incidence of enteric diseases (Hollister *et al.*, 1989, 1990), especially in conjunction with rations having a high starch content (Nieves-Delgado *et al.*, 1992), increases weight gain (Gippert *et al.*, 1992; Yamani *et al.*, 1992; Ayyat *et al.*, 1996) and improves feed conversion (Hollister *et al.*, 1989, 1990). Similarly to Luicke *et al.* (1992), also Kamra *et al.* (1996) found no positive change in performance in spite of increased protein digestibility.

By supplementing the diet with spores from the *Bacillus* genus, Voros and Gaal (1992) found less coliform bacteria in the gut but no effects on growth, similarly to the results obtained by Maertens *et al.* (1994) in the post-weaning phase. In the latter trial, however, a slight improvement (2.3%) was detected in the conversion ratio together with higher weight gain in the pre-weaning phase. Instead, Zoccarato *et al.* (1995) were able to improve weight gain, feed conversion ratio and nutrient digestibility by supplementing the diets with *B. subtilis* and *B. licheniformis*.

The addition of 0.15% live cells of *S. cerevisiae* to the feedstuff made it possible to reduce mortality and increase liveweight both at the time of weaning and after 70 d; at a level of 1% the results were less good (Maertens and De Groote, 1992).

The contemporary administration of 0.5 g/l of acidifiers and microorganisms (Acid Pack 4 way) increases the fermentation activity in the caecum (Kermauner and Strucklec, 1996), thus accounting for better raw fibre digestibility and improved weight gain as found by Yamani *et al.* (1992). However, a drop in performance was observed when the level of treatment was increased to 2 g/l.

New additives for ruminants

Calves

The results reported in the literature about the use of Lactobacilli are not homogeneous; several studies found no improvement in health status and performance following the administration of lactic bacteria (Jenny *et al.*, 1991; Harp *et al.*, 1996). McCormick (1984), reviewing results obtained in the USA with different strains of *Lactobacillus*, noted that only 2 of 10 experiments were positive.

Higginbotham and Bath (1993) and Cruywagen *et al.* (1996) found a non significant improvement in growth rate only in the first 2 weeks after birth in calves fed *Lactobacillus*-supplemented diets.

On the other hand some researchers obtained better performances (Abe *et al.*, 1995) and health status (Abe *et al.*, 1995; Abu-Tarbush *et al.*, 1996) in calves following the addition of *Lactobacillus* to the diet (Table 9).

Table 9. Performance and incidence of diarrhoea in calves fed probiotics with or without anitibiotics (From Abe *et al.,* 1995)

Item	Antibiotics† added to the starter diets			No. antibiotics added	
	Bifidobacterium pseudolongum	*Lactobacillus acidophilus*	Control	Probiotics	Control
No. of calves	15	15	15	10	9
Final weight (kg)	79.3	77.2	71.8	79.9	73.4
Weight gain (kg)	31.8[b]	30.9[b]	25.4[a]	40.5	36.2
Feed/gain	2.1[a]	2.07[a]	2.37[b]	1.59	1.64
Fecal score††	0.19	0.16	0.23	n.r.	n.r.
Diarrhoea cases	n.r.	n.r.	n.r.	1[a]	7[b]

†Colistin sulphate 20 g/t and Zn-bacitracin 4.2 x 10^6 units/t
††Fecal score: 1 = normal; 2 = soft, 3 = scours
a,b: P<0.05

Oligosaccharides, too, have been used in calves with positive results (Webb *et al.,* 1992; Newman *et al.*, 1993; Quigley, 1996; Quigley *et al.,* 1997) (Table 10).

Table 10. Body weight (BW) gain, incidence of scours, dry matter intake (DMI) and feed efficiency of calves fed milk replacere (MR) containing antibiotics† (AB) or galactosyl-lactose (GL) for 26 days (From Quigley *et al.,* 1997)

Item	Treatments			Contrast	
	MR	AB	GL	MR *vs* AB	MR *vs* GL
No. of calves	32	32	32	-	-
BW gain (g/d)	125	177	197	0.07	0.02
Fecal score††	2.27	2.07	2.06	0.07	0.08
DMI (g/d)	475	474	475	NS	NS
BW gain/DMI (g/kg)	282	399	443	0.07	0.08

†138 mg/kg oxytetracycline and 276 mg/kg neomycin
††Fecal score: 1 normal to 4 = sever scours

On the whole, the results obtained by adding probiotics to artificial milk were indeed affected by the environmental conditions, since significant improvements could be achieved when the animals were exposed to stress (Schwab *et al.,* 1980; Seymour *et al.,* 1995), while in normal environmental conditions no improvement was found after the addition of non-conventional sugars to the diet (Jenny *et al.,* 1991; Morrill *et al.,* 1995). As previously reported, by supplementing the diets of calves with lactic acid bacteria, Abe *et al.* (1995) were able to increase performance and reduce scouring, particularly when no antibiotics were fed to the animals.

Adult ruminants

In adult ruminants, antimicrobial agents are used as growth promoters in fattening animals for the prevention of ruminal acidosis and, to a lesser extent, for the control of caecal fermentations; several active ingredients have been approved by the EU (Virginiamycin, Flavomycin, Na-Monensin). Until a few months ago, avoparcin was also allowed and its use extended to dairy cows; subsequently it was banned because of alleged interactions with vancomycin. Such molecules mainly perform their function in the rumen by modulating fermentations and allowing the host animal to achieve greater efficiency, with increased propionate content and reduced lactic acid concentration. Lower protein degradation in the rumen and less CH_4 were also observed. These results are accompanied by other effects like reduced fibre breakdown or reduced protein synthesis in the rumen.

It is, however, possible to manipulate certain biochemical events and the microbial composition of the rumen by means of a probiotic approach. Feeding steers with rations added with cells from *S. cerevisiae* reduces the production of lactate and stabilizes the pH (Williams *et al.,* 1991): this is most likely due to a stimulating action on the bacteria which use lactic acid, e.g., *Selenomonas ruminantium* (Nisbet and Martin, 1991) or *Megasphaera elsdenii* (Rossi *et al.,* 1995); the addition of malic acid to the diet of fattening steers also stabilizes the ruminal pH and improves production performance (Streeter *et al.*, 1994), probably by stimulating *S. ruminantium* (Nisbet and Martin, 1991; Callaway and Martin, 1997) or *M. elsdenii* (Rossi *et al.,* 1995; Chaucheyras *et al.,* 1996). An increase in the use of lactate in *S. ruminantium* can be obtained by using products based on *Aspergillus oryzae* (Nisbet and Martin, 1990). Several studies have pointed to a faster breakdown of the raw fibres in rations supplemented with yeast cultures (Williams *et al.,* 1991; Carro *et al.,* 1992) or *A. oryzae* (Gómez-Alarcón *et al.,* 1990, 1991). They also found increased acetate concentrations in the rumen (Piva *et al.,* 1993), probably due to increased growth rate among the cellulosolytic microflora (Dawson *et al.,* 1990; Callaway and Martin, 1997).

According to Yoon and Stern (1995) (Table 11), the administration of *S. cerevisiae* or *A. oryzae* causes an appreciable improvement in the growth of calves and steers.

Table 11. Effects of *Saccharomyces cerevisiae* or *Aspergillus oryzae* on performances of growing cattles (variation compared to control) (Modified from Yoon and Stern, 1995)

Animal	Dosage (g/d)	Positive trials on total studies	Dry matter intake (%)	Weight gain (g/d)	Feed/gain (%)
S. cerevisiae					
Calves	28.6 ± 51.3	7/8	+6.2	+93.7 (12.1%)	-6.2
Bulls	9.2 ± 1.2	2/2	+4.3	+30.0 (1.9%)	-2.4
Steers	109.5 ± 140.7	2/2	+6.4	+105.0 (8.04%)	-6.0
A. oryzae					
Steers	90 - 113	2/2	n.a.	+45 (4.8%)	n.a.
Calves	0.5 - 3	2/2	+5	+55 (10.5%)	-4.61

n.a.: data not available

The ruminal bacteria have different nutritional requirements in terms of energy and nitrogen (Cotta, 1993; Stewart and Bryant, 1988), peptides (Chen *et al.,* 1987), amino acids and organic acids (Russell and Sniffen, 1984; Masoero *et al.*, 1992). The use of amino acids and organic acids changes the ruminal fermentation profile and microbial growth both *in vitro* (Stack and Cotta, 1986; Masoero *et al.*, 1992; Crepaldi *et al.*, 1994; Barbour *et al.,* 1996) and *in vivo*, with positive effects on the use of feedstuffs and animal weight gain (Masoero *et al.*, 1995; Streeter *et al.*, 1994). In sheep, lactose supplementation in a ration containing corn silage caused an increase in the duodenal flow of microbial proteins (Chamberlain *et al.*, 1993). It is therefore likely that the growth of some microbial groups can be increased to the detriment of others by adequately administering sugars, amino acids or organic acids. Morvan and Fonty (1995) found that the addition of xylose promotes the growth of an acetogenic bacterium which is capable of producing acetate starting from H_2 and CO_2, which would reduce CH_4 production without resorting to antibiotics; similar results were obtained by Chaucheyras *et al.* (1995) using yeast cultures.

Conclusions

The concerns about the possible effects of antimicrobial agents used as growth promoters in animal breeding in terms of increasing antibiotic resistance towards both human and animal pathogens, requires the adoption of an entirely different approach to growth promoters.

Several practical possibilities have been advanced: from rationalizing the use of pro- and prebiotics to adopting nutritional strategies which adequately enhance the immunity and control features of the secretory factors normally found in feedstuffs. The rations shall have to be designed so as to feature precise pharmacological characteristics in order to help minimize the use of conventional drugs in the prevention and treatment of diseases.

Futures developments

Ruminants

Use of rumen-protected oligosaccharides (fructans and mannans) to modulate the microbial activity of the gut. In the rumen, the administration of non-conventional sugars or oligosaccharides could play a certain role on two occasions:

(i) Increased competitiveness between genetically modified ruminal microorganisms.

(ii) Increased competitiveness between important ruminal microorganisms which are usually present in small numbers and administered orally.

(iii) Stimulation of the growth of microorganisms which occupy special ecological niches such as the acetogenic bacteria which use H_2 and reduce CH_4 production. Such bacteria may use xylose, a sugar which is used relatively little by the dominant microbial species. The reduction in CH_4 emissions is an important objective in controlling the environmental impact of animal breeding farms.

Also the administration of trace elements like Cu and Zn, in highly available forms (chelates), adequately protected against ruminal fermentation and performing an antibacterial (Cu), immunostimulating (Zn) or antioxidating (Se) action, might significantly contribute to the modulation of intestinal fermentations.

Monogastrics

Special carbohydrates should be used which selectively stimulate the lactic microflora available in the gut in order to limit both putrefaction and the release of toxic agents.

(i) New intestinal microorganisms should be isolated which can effectively compete with pathogens.

(ii) The polymerization degree of different oligosaccharides should be studied in order to assess the most effective one in the stimulation of the autochthonous intestinal bacteria.

(iii) The probiotic effect of lectins derived from garlic, banana and shallot should be further investigated although interesting results have already been obtained (Koshte *et al.*, 1990; Kaku *et al.*, 1992; Mo *et al.*, 1993).

(iv) Study of other molecules like L-fucose, N-acetyl-D-glucosamine and N-acetyl-D-neuraminic acid able of preventing intestinal walls pathogens adhesion.

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