Strategies for the development of antibiotic-free diets for piglets

J.-P. Lalles

COMPENDIO

Estrategias para el desarrollo de dietas sin antibióticos para lechones

El artículo presenta brevemente el contexto y la perspectiva histórica de la interdicción de utilizar antibióticos en alimentación animal en Europa. Se presentan primero los datos más recientes de la fisiopatología de los desórdenes al destete de los lechones y de los factores que los causan. Después se presentan los modos de acción complejos y los efectos de los antibióticos incorporados en los alimentos como factores de crecimiento y su eficiencia para el lechón al destete. Se mencionan también datos sobre los efectos del cobre y del zinc. Se estudian los modos generales de acción y los resultados técnicos obtenidos por una serie de alternativas al uso de antibióticos, incluidos algunas proteínas particulares y aminoácidos, acidificantes, enzimas, microorganismos, y diferentes preparaciones vegetales. El artículo concluye sobre la necesidad de apreciar mejor el potencial de estas alternativas utilizando criterios científicos más amplios e indicadores de salud más diversificados en las áreas de la fisiología, la bacteriología y la inmunología.

Palabras claves: lechones, destete, desórdenes intestinales, antibióticos, sustancias alternativas.

ABSTRACT

This paper briefly presents the context and historical perspective of the ban on in-feed antibiotics in Europe. After having put some light on recent data on the patho-physiology of post-weaning disorders and risk factors in young pigs, the paper recalls the complex modes of actions and the effects of in-feed antibiotics on growth performance and feed efficiency in the post-weaned piglet. Technical data are also provided on copper and zinc. General modes of action and technical results dealing with an array of alternatives including particular proteins and amino acids, acidifiers, enzymes, micro-organisms and various plant preparations are examined. The paper concludes on the need to better appreciate potential alternatives on larger scientific grounds and with more diversified health indicators in the fields of gut physiology, bacteriology and immunology.

Key words: pig, weaning, gut disorders, in-feed antibiotics, alternative substances

INTRODUCTION

Growth promoting antibiotics have been introduced in feed for farm animals in the 1950s. In the post-World War II context, they contributed largely to the development of intensive animal production through improved animal performance and health status, and overall lowered production costs. Benefits for consumers were increased availability and lowered prices of meat products.

The problem of selection of bacterial resistance to antibiotics in animals and risk of antibio-resistance transfer to humans was raised in Europe at the end of the 1960s (Swann Committee, 1969). Then, the ban on in-feed antibiotics started in 1986 in Sweden and progressively spread out to the rest of Western Europe, with a generalized ban in the European Union planned for 2006.

This evolution in the recent years has been mostly due to increased citizen-consumer concerns for better quality products in a context of perceived general degradation of farm practices. This has been revealed by major problems dealing with environmental pollution (nitrogen, phosphate, metals, pesticides, herbicides, etc.), animal feed adulteration (dioxins) and the Public Health consequences of animal protein incorporation into animal feed (Bovine Spongiform Encephalopathy (**BSE**) in animals and Creutzfeld-Jacob disease in humans). This situation has also been a challenge among the scientific community and professionals of animal production to design and

Institut National de la Recherche Agronomique (INRA), UMR-VP
65 rue de Saint-Brieuc, 35042 Rennes cedex, France; lalles@roazhon.inra.fr

promote safe in-feed antibiotic alternatives. However, technical and economical advantages still need to be demonstrated in many cases.

The present paper focuses on young piglets in the post-weaning (**PW**) period because 1) it is a critical period with growth check and gut disorders, 2) in-feed antibiotics proved to be more effective in young than in older animals, 3) antibiotic withdrawal increased the incidence of diarrhoea post-weaning in pigs, and 4) already evaluated alternatives or strategies have not completely solved this problem. Animal health will be analysed at the gut level and with regard to dietary alternatives. However, the purpose of this paper is not to review all the abundant literature available on alternatives to in-feed antibiotics. Rather, it is aimed at highlighting the various options taken over the last decade, the technical results obtained, the new questions raised and the recent research orientations developed to answer these questions.

IN-FEED GROWTH PROMOTING ANTIBIOTICS

Background and historical perspective of the ban in Europe

Antibiotics used in feed for animals initially comprised avoparcin, tylosin, spiramycin, avilamycin, bacitracin, virginiamycin, salinomycin, carbadox and olaquindox, most of them acting on Gram+ bacteria (Table 1). The progressive ban on in-feed growth promoting antibiotics in Europe has been mainly motivated by the increased development of microbial resistance to antibiotics in human medicine. This could be influenced by the antibiotic-driven selection of resistant microorganisms in farm animals and their transfer to humans or by the presence of antibiotic residues in human food of animal origin. The motivation towards the ban of quinoxaline substances (carbadox and olaquindox) was because of their genotoxic and carcinogenic properties demonstrated in laboratory animals and the increased risk for exposed workers.

This movement started in Sweden in 1986 when the use of in-feed antibiotic substances was banned (Table 2). Nine years later (1995), Denmark prohibited the use of avoparcin as an attempt to limit the occurrence of microbial resistance to another glycopeptide, vancomycin, used in human medicine. Denmark was followed by Germany in 1996. This lead the European Union (E.U.) in 1997 to suspend the use of avoparcin in animal feed for two years. Year 1998 was decisive in this process since, in January, Finland banned tylosin and spiramycin. Soon Denmark did so for virginiamycin and also banned all antibiotic substances in feed for fattening pigs. At the end of 1998, the E.U. came to the decision of banning six substances (bacitracin, tylosin, spiramycin, virginiamycin, carbadox and olaquindox) from 1999 onward. This also hold true for avoparcin whose suspension was not re-examined. The overall ban of growth promoter antibiotics was decided in 1999 in Switzerland and in 2000 in Denmark. Two antibiotics (avilamycin, salinomycin) are still in use in the E.U. but an overall ban is planned for 2006.

Consequences of the ban on in-feed antibiotics

A direct consequence of the ban on in-feed antibiotics has been an increased consumption of antibiotics as therapeutics in all these pioneer countries. In Sweden, this increase appeared two years after the ban and lasted for nearly six years (1988-1994) before it started to decline (Mudd *et al.*, 1999). This phenomenon seemed to be shorter in Switzerland (Wenk, 2002) but the period of observation is still short in this country.

Increased therapeutic use of antibiotics resulted in part from outbreaks of post-weaning diarrhoea (**PWD**) in piglets. For example, in Sweden after 1986, the incidence and severity of PWD doubled, mortality

Table 1. Growth-promoting antibiotics used in animal feed in Europe before the ban.

Name	Class	Spectrum	Antimicrobial mode of action
Avilamycin	oligosaccharide	Gram+	protein synthesis inhibition
Avoparcin	glycopeptide	Gram+	cell wall synthesis inhibition
Bacitracin	peptide	Gram+	cell wall synthesis inhibition
Carbadox	quinoxaline	broad	DNA synthesis inhibition
Olaquindox	quinoxaline	broad	DNA synthesis inhibition
Salinomycin	polyesther	Gram+	membrane alterations
Spiramycin	macrolide	Gram+	protein synthesis inhibition
Tylosin	macrolide	Gram+	protein synthesis inhibition
Virginiamycin	streptogramin	Gram+	protein sunthesis inhibition

(adapted from Anderson *et al.* 2000)

Table 2. Simplified chronology of the ban on in-feed antibiotics in Europe.

Date (Mo)	Country	Main decisión
1986	Sweden	ban on all in-feed antibiotics
1995 (May)	Denmark	ban on avoparcin
1996 (Jan)	Germany	ban on avoparcin
1997 (Jan)	E.U.	suspension of avoparcin for two years
1998 (Jan)	Finland	ban on tylosin and spiramycin
	Denmark	ban on virginiamycin
		ban on in-feed antibiotics for fattening pigs
1998 (Dec)	E.U.	ban on bacitracin, tylosin, spiramycin and virginiamycin
		suspension of avoparcin not re-examined
		ban on carbadox and olaquindox
1999	Switzerland	ban on all in-feed antibiotics
2000	Denmark	ban on all in-feed antibiotics
2006 (planed)	E.U.	ban on all in-feed antibiotics

E.U.: European Union

increased by 1.5 points and growth performance decreased, leading to an age at 25 kg bodyweight increased by 5 to 6 days (review by Thomke and Elwinger, 1998a). Reduced growth performance and feed utilization efficiency have also lead to increased volumes of pig waste effluents, with known negative consequences on the environment in areas of intensive production. Despite increased use of therapeutic antibiotics, the problem of PWD has been usually postponed and not totally solved.

A rise of above 8% in production costs was anticipated from these sanitary and technical changes. The actual economical consequences of the antibiotic ban are, however, difficult to evaluate precisely because of the much wider consequences of the 'mad cow' (BSE) and Foot and Mouth Virus diseases which deeply affected European meat markets during this recent period.

PATHOPHYSIOLOGY OF POST-WEANING DIARRHOEA AND RISK FACTORS

Pathophysiology of post-weaning diarrhoea

In-feed antibiotics have long been used in piglets to overcome the so-called PWD syndrome. The weaning transition is a complex period during which the piglets have to cope with 1) abrupt separation from their mother, 2) mixing with other litters in a usually new environment and, 3) switch from highly digestible (liquid) milk to less digestible and more complex solid feed. Weaning at an early age (between 21 and 28 d) as in intensive production systems has probably exacerbated the level of general stress in these immature animals.

Weaning is usually associated with low and variable feed intake resulting in a transient growth check. Also, intestinal tissue alterations including changes in villus/crypt architecture, depressed activities of many digestive enzymes of the brush border membrane have been documented (review by Pluske *et al.*, 1997). Pathogens (*Escherichia coli* and also rotaviruses) have been most often considered as the primary aetiological factor in PWD. The first hypothesis put forward to explain intestinal damage at weaning has been an adverse immune response to dietary antigens (reviews by Lallès and Salmon, 1994 ; Stokes *et al.*, 1997). Although specific antibodies in plasma were evidenced and that alterations were less pronounced with low-antigenic soya proteins (Lallès and Salmon, 1994), McCracken *et al.* (1998) came to the conclusion that inflammation due to dietary antigens might be secondary to that induced by anorexia.

The second hypothesis is that the lack of intestinal stimulation during anorexia is a primary factor of intestinal inflammation. McCraken *et al.* (1998) showed that inflammatory-T cell numbers and the matrix metallo-proteinase stromelysin increased during the first 2 d post-weaning, at the time of villus atrophy. In 21-d piglets, we observed major morphological, enzymatic and functional changes at the intestinal (jejunum) level after a 48-h fast : 50%-reduction in jejunal villus height, doubling of net ion secretion and three-fold increase in paracellular permeability (J.P. Lallès, G. Boudry, C. Favier, L. Montagne, I. Luron, C. Piel, B. Sève, unpublished data, 2001). During this fast, the expression of genes corresponding to various pro-inflammatory cytokines including IL-1β, IL-6 and

TNF-α was increased in these tissues (I. Oswald, S. Pie, J.P. Lallès, unpublished data, 2002), as were concentrations of particular Heat shock proteins (HSP 27 and HSP 70 ; David *et al.*, 2002) known to protect cells from various stress.

The large intestine has been poorly studied with regard to PW gut health despite its increasing role in dietary component fermentation and volatile fatty acid and mineral absorption after weaning (van Beers-Schreurs *et al.*, 1992). The fermentative capacity of the microbiota takes 2 to 3 weeks to develop and approximately equal amounts of organic acids are produced in the various segments of the gut 2 weeks PW (Jensen, 1998). However, lactic acid dominates in the stomach and small intestine while VFA (acetate >> propionate > butyrate) are characteristic of the large intestine (Jensen, 1998; van Beers-Schreurs *et al.*, 1998). Since these components (especially butyrate) are known to stimulate absorption of minerals (sodium), they probably contribute to solving diarrhoea by re-absorbing excess water coming from the small intestine.

Weaning is also characterized by a high instability of (cultivable) microbial populations along the gut. Jensen (1998) indicated a transient inversion between lactobacilli and coliform bacterial counts in faeces between 2 and 4 d PW in piglets weaned at 28 d of age. This appeared to be true along the gut and more pronounced for bacteria attached to the intestinal epithelium. *E. coli* counts were found 100 to 1000-fold higher 6 d PW as compared to before or 3 weeks after weaning, illustrating the increased susceptibility of weaned pigs to scouring. As enteric bacteria probably play an important role in intestinal inflammation and PW disorders, bacterial diversity and stability during this period need to be addressed by new molecular techniques (Simpson *et al.*, 2000). This will help to understand better the importance of subtle changes in gut bacterial balance and to design new strategies for developing in-feed antibiotic alternatives.

Dietary risk factors associated with post-weaning growth check and diarrhoea

As already mentioned, growth check and PWD are often associated with low feed intake and usual inadequate water intake (Thacker, 1999). Episodes of over-feeding are also a risk factor. An intesresting approach to improving this situation is undoubtedly liquid feeding (review by Brooks *et al.*, 1999). In addition to stimulating (and regulating) intake, this has been proven to minimize intestinal tissue alterations and incidence and severity of PWD.

Twenty years ago, (Prohaszka and Baron, 1980) demonstrated that weaner diets with high protein content (21%) and, therefore, high buffering capacity, increased the frequency and severity of PWD. Reducing this level and using synthetic amino acids made part of measures taken by Sweden after 1986 to reduce the sanitary problems with piglets.

Regarding carbohydrates, in particular soluble non-starch polysaccharides, recent investigations conducted in Australia (with guar gum or carboxy-methyle-cellulose) have shown these compounds to favour intestinal proliferation of pathogenic *E. coli* and reduce growth performance in weaned piglets (McDonald *et al.*, 1999, 2001). Also addition of lupin or wheat favoured the development of the spirochete *Brachyspira hyodysenteriae* in the large intestine of piglets (Siba *et al.*, 1996 ; Pluske *et al.*, 1998). However, attempts to reproduce the models of colibacillosis in other countries such as Canada or Denmark (Hampson *et al.*, 2001) and France (J.P. Lallès, C. Favier, C. Piel, B Sève, unpublished data, 2002) have failed. This suggests that other dietary ingredients or pig gut flora are critical. Different types of carbohydrates such as resistant starch (amylomaize, potato, etc.) and fibre sources (wheat bran) have usually reduced PW gut disorders (review by Williams *et al.*, 2001).

MODES OF ACTION OF IN-FEED ANTIBIOTICS ON GROWTH AND TECHNICAL RESULTS IN PIGLETS

Modes of action of in-feed antibiotics on growth

The aim of this short section is merely to recall that in-feed antibiotics act on growth in a complex, not yet totally understood, manner. As recently reviewed by Anderson *et al.* (2000), growth-promoting antibiotics are known to modify gut microbial populations and to induce larger responses in young, less mature, animals and in those reared in poor sanitary conditions. The growth-promoting mechanisms of antibiotics are apparently independent from their antibiotic mode of action. Part of the growth promotion may reflect antibiotic-reduced alterations in the gut microbiota. These authors proposed four principal effects of antibiotic-mediated growth promotion at the gut level (Table 3):

1. inhibition of sub-clinical infections
2. reduction of growth-depressive microbial metabolites
3. reduction of microbial use of nutrients, and
4. enhanced uptake and use of nutrients by the host.

Overall, normal flora contribute to a thicker gut wall with reduced absorptive capacity, increase tissue

Table 3. Mode of action of in-feed antibiotic growth promoters.

Microbial		Physiological	
Beneficial bacteria	+	Feed intake	+/- 0
- lactobacilli	+	Gut	
- beneficial *E. coli*	+	- wall tissue mass	-
Adverse bacteria	-	- mucosa turnover	-
- pathogenic *E. coli*	-	- absorption capacity	+
- pathogenic Streptococci	-	- feed transit time	-
- *Clostridium perfringens*	-	Faecal moisture	-
Gut flora		Stress, inflammation	-
- nutrient synthesis	+		
- competition for nutrients	-		
Nutritional		**Metabolic**	
Nutrient, vitamin & mineral absorption	+	Production of ammonia, amines, toxins	-
Plasma nutrients	+	Bacterial cell wall, DNA, protein synthesis	-
Energy & protein retention	+	Faecal fat excretion	-
Gut energy losses	-	Gut urease	-
		Gut alkaline phosphatase	+

(adapted from Thomke and Elwinger, 1998)

turnover and level of inflammation, thus increasing the maintenance requirements of the host. Gut bacteria compete with the host for digestible nutrients in the small intestine. They degrade amino acids and urea leading to the production of ammonia at levels considered as aggressive to the cells, and highly toxic phenolic and aromatic compounds derived from tyrosine and tryptophan degradation. Bacteria are also implicated in luminal deconjugation and dehydroxylation of bile acids, thus producing additional toxic molecules and impairing lipid absorption. The high complexity of in-feed antibiotic action strongly suggests the difficulty to compensate for their withdrawal using a single alternative.

In-feed antibiotics, copper and zinc : technical results in weaned piglets

Thomke and Elwinger (1998) indicated that antibiotics improved growth performance and feed utilisation efficiency by 9 to 30% and 6 to 12%, respectively, depending on the type of antibiotics, feeding and rearing conditions and physiological state of the animals. In a recent literature survey conducted by the French Pig Technical Institute, Gourmelen *et al.* (2002) came the conclusion that on the entire PW period (4 weeks) in-feed antibiotics had an overall positive effect of 10.3 and 6.5% on growth and feed efficiency, respectively (Table 4). When the immediate PW period of 2 weeks was considered, these figures were even higher (15.7 and 7.7 %).

High doses of copper and zinc have also been used for decades to prevent PWD. Mineral salts of copper and zinc were taken into account in this survey by Gourmelen *et al.* (2002). These substances are well known for their antimicrobial effects but there are concerns towards manure-induced pollution and soil toxicity when high doses of metals are permanently used in feed. Requirements for pigs were considered

Table 4. Effects of in-feed antibiotics and metals on growth performance and feed efficiency in young pigs post-weaning (% of values with non-supplemented controls).

	Overall 4-wk PW period				Immediate 2-wk PW period			
Substance	ABG[1]	FCR[2]	*Stat.*[3]	*(n)*[4]	ABG	FCR	*Stat.*	*(n)*
Antibiotics[5]	+10.3	-6.5	*76*	*39-28*	+15.7	-7.7	*0*	*16-3*
Quinoxalines[5]	+16.1	-6.2	*100*	*11-2*				
Copper sulfate 1[6]	+12.6	-3.8	*87*	*10-8*				
Copper sulfate 2[7]	+12.7	-4.0	*56*	*14-9*	+17.3	-6.4	*50*	*13-10*
Zinc oxide 1[8]	+9.3	-2.6	*77*	*13-13*	+15.5	-5.4	*75*	*24-24*
Zinc oxide 2[9]	+7.6	-2.3	*86*	*7-7*				
Zinc oxide 3[10]	+11.2	-2.9	*67*	*6-6*				
Zinc Sulfate[11]	-3.3	-1.3	*0*	*7-7*				

[1] Average bodyweight gain, [2] Feed conversion ratio, [3] pourcentage of studies with statistics having a significant effect ($P < 0.05$), [4] n numbers of retained studies - studies with statistics. [5] substances allowed before September 1999. Doses of metal salts (mg/kg feed) : [6] 90-175, [7] 180-250, [8] > 2000, [9] 2000-2500, [10] 3000, [11] 1500 (adapted from Gourmelen *et al.* 2002)

to be close to 10 and 100 mg/kg feed, respectively (INRA, 1989), but a reappraisial of these requirements is currently under investigation at INRA. Also, legal levels of metals in animal feed have been recently fixed at the EU levels, being for pigs 175 mg (age < 16 weeks) and 250 mg for copper and zinc, respectively. Nevertheless, this survey confirms a important improvement of growth performance (> 12%) and feed efficiency (> 4%) with copper sulfate, this irrespective of the class of doses used (90-175 or 180-250 mg/kg feed). As expected, this effect was larger in the immediate PW period.

Zinc oxide, as expected, had large beneficial effects on both growth performance and feed efficiency over the total PW period. Doses comprised between 2000 and 2500 mg/kg feed had already an important impact during the immediate PW period (15.5 and 5.4% on growth and feed efficiency, respectively). These figures were even larger with higher doses (3000 mg/kg feed) clearly showing a dose-dependent response still at high levels of zinc. Contrasting with these results, zinc sulfate appeared of very limited interest (-3.3 and 1.3% on growth and feed efficiency, respectively). However, the average doses of zinc provided as sulfate were also lower (1500 g/kg feed) in the studies analysed by Gourmelen *et al.* (2002).

ALTERNATIVE SUBSTANCES TO IN-FEED ANTIBIOTICS AND TECHNICAL RESULTS IN WEANED PIGLETS

Alternative substances to in-feed antibiotics are aimed at preventing the development of gut disorders and not at treating diseases. Alternatives have been given various names including 'pronutrients' (Wenk, 2002) or "nutribiotics" (Piva *et al.*, 2002).

Dairy products and other animal proteins

There is no doubt that dairy products including skim milk powder and whey powder and derivatives have favourable effects on feed intake, growth performance, feed efficiency and health in young pigs, due to high digestibility of protein and energy (review by Thacker, 1999). However, dairy products are expensive, a factor limiting their incorporation rates into weaner diets.

Other animal proteins except fish meal are banned in animal feed in the E.U., in connection with the BSE disease and possible transfer to humans through animal products. Nevertheless, plasma proteins in the form of spray-dried plasma revealed to be of high value in diets for piglets. In their review, van Dijk *et al.* (2001) reported that plasma protein incorporated at levels up to 6% lead to large improvements in growth performance (+26.8% on average for 15 studies), mostly due to increased voluntary feed intake (+24.5%), with a modest effect on feed conversion ratio (-3.2%). Incidence and severity of diarrhoea were most often reduced. Effects on growth were mostly explained by the high palatability of plasma proteins. They were of lower magnitude when control diets with vegetable ingredients as compared to dairy products and lower with bovine, as compared to porcine, plasma proteins. Van Dijk *et al.* (2001) provided three other explanations for these positive results : 1) growth stimulated by plasma protein IGF-1 (not demonstrated yet), 2) glycoproteins not linked to the plasma immunoglobulin (Igs) fraction and which have been shown to protect against pathogenic *E. coli* (Nollet *et al.* 1999), and 3) improved immunocompetence through plasma Ig. Recently, Touchette *et al.* (2002) have clearly shown that spray-dried plasma reduced the level of basal activation of the immune system in various organs, as evaluated through gene expression of cytokines like IL-1β, IL-6 or TNF-α. The density of immune cells in the intestinal mucosa was also reduced (Jiang *et al.*, 2000). Challenging plasma-supplemented piglets had various effects. Van Dijk *et al.* (2002a) found no reduced incidence of diarrhoea and mortality following a pathogenic *E. coli* challenge between soyabean/whey-fed and plasma-supplemented piglets. By contrast, piglets fed plasma and immunologically challenged with bacterial lipopolysaccharide displayed an over-response of their immune system leading to intestinal damage (Touchette *et al.*, 2002). Work is now in progress to produce and evaluate (pig) plasma hyperimmunised against pathogenic strains of *E. coli* (van Dijk *et al.*, 2002b).

This immunological approach has already provided successful results in piglets fed with chicken egg (yolk) hyper-immunised against particular fimbrial antigens purified from pathogenic strains of *E. coli* (Imberechts *et al.*, 1997; Marquardt *et al.*, 1999). However, this approach may have undesirable side-effects such as delaying pigs' own immune responses and postponing the onset of disease in herds (Imberechts *et al.*, 1997).

Amino acids and polyamines

Among non-essential amino acids, glutamine and glutamate have been the most studied in connection with gut health both in humans and animals. These amino acids are important fuels for intestinal cells.

Supplementation of diets with glutamine or glutamate prevented intestinal villous atrophy and improved feed efficiency after weaning (Wu *et al.* 1996 ; Ewtushick *et al.*, 2000). Arginine also prevented villus atrophy (Ewtushick *et al.*, 2000).

The anti-secretory factor (ASF) is a protein that provides protection against diarrhoeal diseases and intestinal inflammation (review by Lange and Lonnroth, 2001). ASF is present in in pigs and, interestingly, has been shown to be at low levels in plasma following weaning. Amino acids alanine and glycine added to weaner diets proved to be effective in stimulating production of ASF and improving growth performance and reducing incidence of diarrhoea post-weaning (Goransson, 1997).

Polyamines are important nutrients implicated in the maturation of the gut (review by Peulen *et al.*, 2000). Polyamine supplementation in piglets had either favorable effects on both growth and intestinal integrity (Grant *et al.* 1990) or were detrimental to intestinal growth (Ewtushick *et al.*, 2000). These discrepancies suggest additional work is needed.

Acidifiers

Modes of action. The rationale for acidifying the diets for piglets is that : 1) gastric acid is low at weaning thus limiting endogenous enzyme activation and subsequent digestion, 2) weaner diets have substantial buffering capacity which favours bacterial growth and, 3) acids are known to display antibacterial properties. Although the technical results are often improved (see below), the mechanisms seem to depend on various factors including the type and dose of acid. In their review, Roth and Kirchgessner (1998) provided optimal doses for various organic acids (Table 5). Digesta pH was usually decreased in the stomach but it was either unchanged or even increased in the small intestine (Roth and Kirchgessner, 1998; Jensen, 1998). From the microbial standpoint, Roth and Kirchgessner (1998) indicated that formic acid acted against various bacteria (*Bacillus, E. coli, Salmonella*) and yeasts without affecting *Lactobacilli* counts. Lactic acid was indicated to decrease haemolytic *E. coli* counts in the stomach, and increase lactobacilli and yeast counts and decrease coliforms along the rest of the gut (Jensen, 1998). Effects on bacterial populations are not always so clear.

Technical results. Among alternatives, acidifiers have been the most studied (> 100 studies analysed in the survey by Gourmelen *et al.*, 2002). A high variability of results was observed which could be accounted for by various factors including the type of acidifier, diet and experimental condition used. Mineral acids appeared to have detrimental effects on growth with no effect on feed efficiency (Table 6). It is probably the reason why they are not used very much. By contrast, organic acids (all forms together) improved growth performance and feed efficiency on average by 4.6 and 3.3%, respectively, for the entire PW period, with significant effects in half of the studies. These figures were much higher immediately after weaning (11.7 and 7%). Among organic acids (or their salts), tri-acids (citric, tartric) has lower effects than mono-acids (acetic, benzoic, formic, lactic, propionic, sorbic) or di-acids (malic, fumaric). Acidifier complexes were also evaluated and proved to be better (8.2 and 3%) than single/simple forms of organic acids.

Enzymes

Modes of action. Exogenous enzymes are added to diets either to supplement those that are already present in the gut or to provide hydrolytic capacity that is totally absent. Most enzymes are directed towards dietary constituents with known anti-nutritional properties or those limiting the overall access to

Table 5. Organic acids, pKa values and optimal doses in feed for piglets.

Acid	Acid function	pKa	optimal dose (%)
Formic[1]	1	3.8	1.2
Acetic	1	4.8	
Propionic	1	4.9	
Butyric	1	4.8	
Lactic	1	3.8	1.6
Sorbic	1	4.7	2.4
Fumaric	2	3.0, 4.4	2.0
Malic	2	3.4, 5.1	2.4
Tartric	2	2.9, 4.2	
Citric	3	3.1, 4.8, 6.4	4.6

[1] also used as sodium formate (dose 1.8%) and calcium formate (1.3%) and potassium diformate (1.8%).
(adapted from Roth and Kirchgessner, 1998).

nutrients (review by Bedford and Schulze, 1998). Animal responses to exogenous enzymes tend to vary according to numerous factors among which animal age and health status, and interaction with other dietary constituents (Bedford and Schulze, 1998). Owing to these variations, the mode of action of a number of enzymes is still debated.

Soluble non-starch p"olysaccharides (arabinoxylans and β-glucans of cereals) are responsible for decreased feed intake, lowered digesta passage rate due to increased luminal viscosity, altered intestinal tissues and reduced absorption of nutrients and water, and disturbed microbial balance, especially in birds (Bedford and Schulze, 1998). Appropriate enzyme supplementation proved to restore intestinal function, growth performance and health in birds. Young pigs appear to be less sensitive to viscosity.

Technical results. From the literature review by Thomke and Elwinger (1998c) in weaned piglets, it appeared that after enzyme supplementation, growth performance was increased by 3.4% and feed efficiency by 6% on average. Variations were large. Effects were partly attributable to improved digestibility of protein and energy. ß-Glucans had substantial effects on growth and reduced incidence of diarrhoea was sometimes reported (Simon *et al.*, 2001).

In the survey by Gourmelen *et al.* (2002), studies were conducted with various enzymatic preparations including hemicellulases and enzymes complexes (Table 6). The former displayed favorable effects on the overall PW period (7.6 and 5.7% for growth and feed efficiency, respectively). By contrast, enzyme complexes, although more studied, did not appear to present a clear beneficial effect.

Probiotics

Modes of action. Probiotics are live microorganisms which beneficially affect the host by improving its intestinal microbial balance (Zimmermann *et al.*, 2001). The modes of action are still partly enigmatic. The anti-pathogenic mechanisms has been classified as bacterial antagonism, competitive exclusion and immune stimulation. The first two mechanisms seem to involve various inhibitory substances including organic acids, hydrogen peroxide and bacteriocins.

In young pigs, supplementation of sows' diets with probiotic bacteria proved to be beneficial to piglets both before and after weaning. *Bacillus cereus* (var. *toyoi*) was able to depress the growth of enterobacteria in suckling pigs while a reduction in the variability of growth of bacteria was usually observed in nearly all intestinal segments of pigs before and after weaning (Jadamus *et al.*, 2000, 2002). *Lactobacillus casei* (in association with maltodextrin or fructo-oligosaccharide) were able to inhibit *E. coli* adhesion to jejunal mucosa and to stimulate phagocytic activity of blood cells (Bomba *et al.*, 1999). *Lactobacillus paracasei* stimulated intestinal growth of lactobacilli and bifidobacteria (Nemcova *et al.*, 1999). Lactobacilli strains isolated from piglet small intestine had good

Table 6. Effects of alternatives to in-feed antibiotics on growth performance and feed efficiency in young pigs post-weaning (% of values with non-supplemented controls).

	Overall 4-wk PW period				Immediate 2-wk PW period			
Substance	ABG[1]	FCR[2]	*Stat.*[3]	*(n)*[4]	ABG	FCR	*Stat.*	*(n)*
Acidifiers								
mineral acids	-8.5	-0.2	*17*	*6-6*				
organic acids	+4.6	-3.3	*42*	*75-63*	+11.7	-7.0	*53*	*77-49*
organic acid complexes	+8.2	-3.0	*50*	*41-40*				
Enzymes								
hemicellulases	+7.6	-5.7	*100*	*5-3*				
enzyme complexes	+1.6	-1.0	*25*	*22-16*				
Microorganisms								
bacteria	+5.6	-3.0	*13*	*24-15*	+18.0	-7.3	*50*	*12-6*
yeast	-0.3	+0.3	*7*	*14-14*	+12.0	+5.3	*33*	*3-3*
Plant preparations								
all types[5]	+1.8	-0.6	*12*	*42-41*				
essential oils	+3.2	-6.6	*50*	*8-8*				

[1] Average bodyweight gain, [2] Feed conversion ratio, [3] pourcentage of studies with statistics having a significant effect ($P < 0.05$), [4] n number of retained studies – studies with statistics. [5] Include aromas, oligosaccharides, essential oils and various plant extracts (adapted from Gourmelen *et al.* 2002).

ability to attach epithelial cells and inhibit adhesion of enterotoxigenic *E. coli* (Jin *et al.*, 2000a). This was also true for a particular strain of *Enterococcus faecium* (Jin *et al.*, 2000b). Other examples in rats demonstrated that particular strains of probiotics can modulate antigen uptake by the intestinal mucosa (Pessi *et al.* 1998) or protect intestine from damage (induced by zinc deficiency; Mengheri *et al.*, 1999).

Technical results. Probiotic bacteria had a favorable effect on both growth performance and feed efficiency (5.6 and 3%, respectively) during the overall PW period (Table 6). However, this was significant in two out of 15 studies only. Yeast (*Saccharomyces*) had little effect on this overall PW period. It is of note that both probiotic bacteria and yeast displayed more favorable effects immediately post-weaning, with still an advantage for bacteria (18 and 7.3% *versus* 12% and -5.3% for yeast).

Prebiotics and plant preparations

Modes of action. Prebiotics are specific dietary ingredients that will improve the activity or development of bacteria beneficial to the host. Most of them are natural constituents from plants including legume grains and cereals. Non-digestible oligosaccharides (NDO) which include a variety of molecules (Table 8) are substrates specific for particular bacterial strains (Table 7). These compounds may increase the barrier effect against infections by enteric pathogens through stimulation of lactic acid production in the small intestine and volatile fatty acids in the large intestine. Also, fermentation of NDOs may occur in different sections of the gut depending on the type of NDO. In their review, Williams *et al.* (2001) indicated that fructo-oligosaccharide (FOS) was fermented up to 90% at the end of the small intestine, trans-galacto-oligosaccharide (TOS) was only at 30% and values for galacto-oligosaccharide (GOS) varied depending of the botanical origin (65 and 90% in the case of pea and lupin, respectively).

Many different plant products including herbs, spices and botanicals have recognised antimicrobial and anti-oxidative properties (among others) and they may positively influence feed intake, gastro-intestinal secretions, the microflora and the immune system (Wenk, 2002). Owing to the large diversity of compounds and modes of action, Wenk (2002) concluded that they still need proper screening and evaluation.

Technical results. Overall, in the survey by Gourmelen *et al.* (2002), plant preparations

Table 7. Some non digestible oligosaccharides.

Name	chemical structure[1]	number of sugar units	type of link
Raffinose	Fru, Gal, Glu	3	
Stachyose	Fru, Gal, Gal, Glu	4	
Iso-malto-oligosaccharide (IMO)	Glu (iMal)n	2-4	alpha 1-6
Mannan-oligosaccharide (MOS)	(Mannan)n	2-8	alpha 1-6
Galacto-oligosaccharide (GOS)	Glu (Gal)n	2-5	beta 1-6
Lactulose	Gal, Fru	2	beta 1-4
Xylo-oligosaccharide (XOS)	(Xylose)n	2-9	beta 1-4
Fructo-oligosaccharide (FOS)	Glu (Fru)n	2-10	beta 1-2
Glu(Fru)	10-50	beta 1-2	

[1]Fru = fructose, Glu = glucose, Gal = galactose, iMal = iso-maltose

Table 8. Bacterial fermentation of some oligosaccharides.

	Oligosaccharide[1]				
Bacteria	FOS	INU	TOS	IMO	LAT
Bacteroides (various species)	+	+	+	+	+
Bifidobacterium spp.	+	+	+	+	+
Clostridium perfrengens	+, -	-, +	-, +	+	+
Escherichia coli	-, +	-	+	-	+,-
Lactobacillus casei	+,-	+	+	-	+

[1] FOS = Fructo-oligosaccharide, INU = inulin, TOS = Trans-galacto-oligosaccharide, IMO = Iso-malto-oligosaccharide, LAT = lactulose (adapted from Zimmermann *et al.*, 1998)

(corresponding to aromas, oligosaccharides, essential oils and various plant extracts) had limited beneficial effect on growth performance (1.8%) with no effect on feed efficiency (Table 6), but the number of studies was limited. However, among plant preparations, so-called essential oils influenced more positively growth (3.2%) and feed efficiency (6.6%), the effects being significant in half of the studies.

Associations of alternatives to in-feed antibiotics

Solutions to replacing in-feed antibiotics may be, at least in part, through association of alternatives. The most famous association is probably between prebiotics and probiotics, also called symbiotics. Various strains of lactobacilli associated with fructo-oligosaccharides or malto-dextrins were able to increase inhibition of *E. coli* adhesion to intestinal mucosa and stimulate phagocytic activity in blood cells of weaned pigs (Bomba *et al.*, 1999; Nemcova *et al.*, 1999). Enhanced positive results have been obtained in pigs when phytase or formic acid were associated with oligo-saccharides (Bolduan, 1999). Lactitol and tributyrin synergistically enhanced the trophic status of intestinal mucosa and reduced histamine levels in the gut of piglets (Piva *et al.*, 2002).

CONCLUSIONS AND FUTURE PROSPECTS

A large array of potential alternatives to-in feed antibiotics have been evaluated so far in various conditions but only a small number have showed significant (although often variable) improvements in health and production parameters in young pigs. Recent investigations in gut patho-physiology suggest that anorexia which is frequently observed immediately after weaning not only leaves the piglets in a catabolic state (McCraken *et al.*, 1995) but also initiates small intestinal inflammation. This, in turn, increases the risk for secondary adverse immune responses to dietary antigens and infections. The period of weaning is also characterized by gut bacterial instability and population diversity changes that may transiently be detrimental.

Among tested alternatives, various acidifier complexes, hemicellulases, probiotic bacteria and organic acids appeared to have the best effects on growth performance and feed efficiency. However, these improvements were usually 30 to 60% lower than those observed using metals (at high doses) or in-feed antibiotics. Collectively, these data suggest that associations of alternative substances need to be studied further in order to have a better chance to reach the effectiveness of metals (high doses) or in-feed antibiotics. Also new strategies based on the use of feed ingredients with various fermentation properties are being evaluated (Williams *et al.*, 2001). Feeding strategies best preventing PW gut disorders in piglets still need improvements in our comprehension of the patho-physiological mechanisms behind and of the modes of action on dietary ingredient associations on gut health. Finally, many other factors including hygiene, management and husbandry levels are relevant to the sanitary risk (Madec *et al.*, 1998) and should be taken into account in integrated strategies.

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