

Review

Gut-trophic feed additives and their effects upon the gut structure and intestinal metabolism. State of the art in the pig, and perspectives towards humans

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Summary. The correct functional development of the gastrointestinal tract is of special importance during the neonatal and weaning phases of reared piglets. Nutrition is obviously a critical determinant in the growth of the gut in the young swine. The mucosal epithelium of the small intestine is reputed anatomically and functionally immature in neonatal pigs, a feature that appears to be exacerbated at weaning, when a colonization of the gut occurs by “new” microorganisms entering the alimentary canal with the solid feed. This frequently exposes piglets to diarrhoeic syndromes and other intestinal disturbances. Functional feed additives, also called nutraceuticals, appear as promising alternative substances to the use of chemotherapeutics as growth promoters in the rearing farm, above all considering the near banning of them by the European Parliament in the view of reducing antibiotic resistance phenomena in human therapies. Several feed additives are available that may play a role in the pig nutritional plan because of their trophic and cyto-protective effects on the gastrointestinal apparatus. Paying special attention to the quantitative consequences (histometry) upon the gut of the examined dietary supplements, this review, even if not fully exhaustive, will focus on the function (and possibly the mechanism/s of action) of certain gut-trophic nutrient substrates. This in turn will sustain the potential use of these substances in human therapy, especially the one directed at resolving intestinal diseases, both in adult and infant ages. In nutritional studies as well as in other biomedical research fields, the swine is an excellent animal model.

Key words: Nutraceuticals, Weaning, Piglet, Gut Histometry, Swine animal model

Introduction

Food is an important stimulus for the growth and maintaining of gastrointestinal mucosa, and, in this respect, gut structure is influenced by the route of nutrient administration, dietary composition of the meal, and availability in it of specific nutrients.

In the lifespan of the pig, above all when reared, the neonatal and weaning phases represent critical periods for both the correct development of the gut and the growth of the young animal (Pluske et al., 1997; Lay et al., 2001).

The correct and timely functional development of the gastrointestinal tract, in which it should be able to sustain growing digestive, absorptive and immune functions, is of particular importance for these adaptable processes correctly occurring. Really, the gastrointestinal tract serves a key functional role in the growth of the young piglet, even if it represents a relatively small fraction of its body weight, in that it is approximately 2% of body weight at birth and increases nearly three fold, to more than 6% two weeks after weaning (Shields et al., 1983; Mitchell et al., 2001). Nutrition is obviously a critical determinant in the functional development and growth of the gastrointestinal tract, and, on the other hand, fasting causes in this species a marked intestinal atrophy, as in humans (Alpers, 2002). In addition to serving as both substrates for oxidative energy and precursors for the synthesis of constitutive and secreted functional proteins, glycoproteins, nucleotides and membrane lipids, nutrients indirectly stimulate the production of endocrine as well as paracrine hormones, growth factors and a variety of metabolites that affect gastrointestinal physiology, with both stimulating and inhibitory effects. The action of dietary nutrients towards stabilized beneficial microorganisms in the intestinal biocenosis cannot finally be ignored, and microorganisms themselves when disrupted may furnish small molecules to be utilized in the functional development of the gastrointestinal apparatus.

The gut development and growth is due to the stimulation associated with oral feeding that largely begins at birth, although some swallowing of amniotic fluid occurs in foetal pigs (Sangild et al., 2000). Ingested nutrients have obviously a major anabolic effect on the neonatal gut (Burrin et al., 2000), but there are also numerous growth factors present in sow's colostrum and milk that are believed to have trophic effects on the gut (Remillard et al., 1998), independently of the presence in them of nutrients.

During the weaning process there are several key nutritional and environmental factors that contribute to important changes in the structure and displayed functions of the gastrointestinal tract (Pluske et al., 1997; Dreau and Lalles, 1999; Burrin and Stoll, 2002; Hedeman et al., 2003; Boudry et al., 2004; Carlson et al., 2004). These factors include: 1) the change in nutrient ingestion, namely from suckling the dam to ingesting feed from a feeder, which, in conjunction with the withdrawal from the sow and mixing with unfamiliar piglets, is directly responsible for a psychological and behavioural stress; 2) the changes in the physical aspect, chemical composition, and sensorial characters of the meal, from a liquid to a dry one, with consequent qualitative and quantitative changes in the gut microflora.

If the altered gut biocenosis is reputed to be responsible for the majority of intestinal pathologies with the frequent occurrence of diarrhoea in weaning piglets (Alexander, 1994), all the cited factors simultaneously interact with each other to produce a reduction in feed intake, which in turn causes a diminished overall mass and mucosal components of the small intestine, with the occurrence of dramatically reduced intestinal villi and crypt hyperplasia (Pluske et al., 1997; Owusu-Asiedu et al., 2003). Villous atrophy, in turn, may cause important limitations in key nutrients needed to maximize the growth of peripheral tissues, such as skeletal muscle.

When, on the other hand, the adaptive phase of weaning may be considered successfully finished at around seven-eight days from the diet substitution, the resumption of a relatively normal feed intake is marked by significant increases in the masses of the small intestine, stomach and large intestine, owing to a higher dry matter intake, and the presence of fibre content, with a function of mechanical stimulus for the gastrointestinal mucosa. Mucin glycoproteins (mucins) represent an important marker of the gut fully acquired functional roles, and their secretion is usually increased after weaning, owing to the increasing complexity of feed (Deplancke and Gaskins, 2001). The production of mucins may be in addition nutritionally significant, because they are not readily digested (Piel et al., 2004) and thus the colonic fermentation of their constitutive essential amino acids and carbohydrates represents an obligatory loss to the animal, even if nourishing useful microbiota.

Mucins are in addition fundamental bricks for the

building of gut barrier function. Actually, the gut serves as a dynamic interface between the external (the farm room) and the internal (the intestinal lumen habitat) environments of the pig, with its primary functions being to digest and absorb feed and to provide a physico-chemical and immunological barrier against possible harmful materials, such as pathogenic microorganisms, toxins, and allergenic macromolecules. Enterocytes populating the mucosa of the small bowel constitute the cell type, which assures the majority of these functions, and are always in a dynamic state. They are constantly being replaced by cells arising from the intestinal glands, with the rate of regeneration matching the normal loss of apoptotic cells at villous epithelium (Tang et al., 1999; Van Dijk et al., 1999). In the large bowel, the constant replacing of enterocytes is present, even if at a lesser extent than in the small bowel, and occurs at the apex of intestinal glands. The mucosal epithelium of the small intestine is regarded as anatomically and functionally immature in neonatal pigs (Pluske et al., 1997), a feature that is exacerbated at weaning, when a colonization of the gut occurs by "new" microorganisms entering the alimentary canal with the solid feed. These are assigned to become commensal microorganisms, but the transformation of some of them in pathogens is not an infrequent event around weaning. When a pathogen enters the alimentary canal, due to its penetration within the gut barrier, it causes defensive modifications that result in an activation of the mucosal immune system and release of pro-inflammatory cytokines (e.g. tumor necrosis factor and interleukins), which have been shown to increase crypt cell proliferation, villous enterocyte apoptosis (Rafferty et al., 1994; Pigué et al., 1999), and synthesis of intestinal acute phase proteins and mucins (Breuille et al., 1998).

Thus, the postnatal period from birth to post-weaning (through weaning) is marked by a substantial increase in gastrointestinal masses and gut proper functional roles assumption. The increases in proteins and glyco-proteins synthesis and cell mass, coupled with increased pathways of cellular metabolism, should translate into increased nutrient needs for the gut. However, studies are infrequent (Torrallardona et al., 2003; Van Nevel et al., 2003) dealing with the possible presence of specific nutrients for this neonatal-weaning transition period, aimed at displaying nutritive roles for both the growing piglets and the growing necessities of their alimentary canals.

Functional feed additives (also called "nutraceuticals") might fall within this argument. Functional feed additives are primarily alternative substances to the use of antibacterial agents and chemotherapeutics during weaning in the rearing farm, as they can adequately stimulate the local defensive responses, and favourably influence resident gastrointestinal microflora, but are also able to improve nutrient digestion and absorption. This in addition appears a very promising possible goal in the view of single EC countries applying the recent Directive

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70/524/CEE (2002/C 329 CE) of the European Parliament regarding the gradual blockage in the use of antibiotics as alimentary additives for food animal species. There is consequently an urgent request for the development of alternative substances to the use of chemotherapeutics, and functional feed, in their variety, may be favourably examined in this respect.

In the past decade, studies have demonstrated that several nutrients are available that may play a role in the pig nutritional plan for their trophic and cyto-protective effects on the gastrointestinal apparatus. The review will focus on the function (and possibly the mechanism/s of action) of certain gut-trophic nutrient substrates, with the perspective that the examined substances may be potential feed additives for optimising the growth, function and health of the gut in weaning piglets. In addition, considering that the swine is an excellent animal model for analysing fasting and malnutrition (Alpers, 2002), and for biomedical research (Swindle, 1998; Bruins et al., 2002; Li et al., 2003; Fan et al., 2004), a deeper knowledge of this argument will possibly contribute to assuring the intestinal health status in humans, above all from a preventive point of view, against a lot of intestinal pathologies, namely those degenerative ones.

This review did not want to be fully exhaustive of this argument, but intended to evidence some especially interesting aspects of the consequences upon the intestinal structure of dietary administration of gut-trophic additives. We are especially concerned in analysing by a morpho-functional point of view these structural aspects, because the evidenced changes (in comparison with control, not-supplemented animals) may in some instances suggest possible ways of action of functional foods, and so elicit interesting multidisciplinary themes of research.

L-Glutamine

Studies during the last decades have demonstrated that glutamine is an amino acid of a special importance from the point of view of determining and guarding normal metabolic processes of the cell (Brooks et al., 1997). This amino acid is indispensable for the optimal growth of most renewing cells and tissues. Although glutamine is usually considered a non-essential amino acid, numerous studies have demonstrated that endogenous glutamine storage and synthesis capability may in some instances be not sufficient to meet the body needs during long-term stress, hypercatabolic and hypermetabolic states, or prolonged starvation (Hammarqvist et al., 1996; Elia and Lunn, 1997; Griffiths et al., 1997; Peng et al., 2004). This has led to a redefinition of glutamine as a “conditionally essential” amino acid.

L-Glutamine has received considerable interest as a gut-targeted nutrient, due to its proposed key role in the maintenance of intestinal structure and function. Glutamine seems to be indispensable as a metabolic fuel

to be fully oxidized by the epithelial layer of intestinal mucosa (Zhou et al., 2001) and is reputed to act in a threefold way: i) it provides nitrogen precursors for mucosal anabolic pathways to maintain intestinal structure and function, ii) it supplies epatocytes with an optimal substrate mix, and iii) it provides citrulline/arginine for the whole organism (Plauth et al., 1999).

As expected, the glutamine necessity, measured as the level of glutamine oxidation up to CO₂, appears to increase with the piglet age (Wu et al., 1994). Micro-anatomical studies in weaning piglets (Ayonrinde et al., 1995) showed that supplementing the diet with 4% crystalline glutamine increased intestinal villi height. When pigs are early weaned, the already described concomitant atrophy of the villi in the jejunum, but not in the duodenum, can be prevented by inclusion of 1% glutamine in the diet (Wu et al., 1994). In 4-d-old suckled pigs, glutamine (at 4.5% inclusion in a total parenteral nutrition experimental model) increased villi height and area in the jejunum, without altering protein or DNA mass (Burrin et al., 1994). This null effect on macro-constituents was also observed with 1-wk-old mini-pigs (Burrin et al., 1991). On the other hand, Li et al. (2003) have found in transplanted (small bowel) pigs that glutamine in total parenteral nutrition significantly improved intestinal disaccharidase activities, villous height, surface area and mucosal thickness, affecting in addition mucosal protein contents. Two recent studies conducted upon weaned piglets showed that 0.5 % dietary glutamine supplementation significantly affected intestinal structure, with higher villi and deeper crypts, in 30 days post-weaning piglets (Domeneghini et al., 2004, 2005; Table 1). The same authors (Domeneghini et al., 2004, 2005; Fig. 1) found that the number of proliferating mucosal cells was higher and the number of apoptotic mucosal cells was decreased in glutamine-treated vs control piglets (Table 1). In addition, dietary glutamine enhanced the numbers of mucosal macrophages and intra-epithelial lymphocytes (IEL),

Table 1. Effects of dietary Glutamine in weaning piglets upon structural aspects of ileum.

	CONTROL	GLUTAMINE	P VALUES
villus height, µm (V)	168.18 ±9.33	207.63±9.33	0.0038
crypt depth, µm (C)	109.97±13.73	166.13±13.73	0.0049
V:C ratio	1.60±0.103	1.25± 0.103	0.0215
Epithelial cells			
(number of cells in 0.015 mm ²)			
apoptosis	90±1.36	84±1.36	0.0109
mitosis	1288±13.89	1344±13.89	0.0159
A:M index	0.069±0.001	0.062±0.001	0.0023
Lymphatic follicles			
(number of cells in 0.015 mm ²)			
apoptosis	78±3.39	58±3.39	0.0012
mitosis	192±2.34	194±2.34	0.58
A:M index	0.32±0.002	0.30±0.002	0.002

thus possibly enabling the young pigs to pass undamaged through a stressful period, as undoubtedly the usually adopted precocious weaning is.

The sometimes discrepant findings in different tracts of the piglet small intestine may reflect differences between them in glutamine metabolic utilization and possibly consequent modified rates of enterocyte-affected proliferation. Really, glutamine appears to affect cell proliferating rates not only indirectly, as a metabolic fuel, but also directly, because it has been shown to possess its own stimulating actions towards proliferation in an *in vitro* study (Blikslager and Roberts, 1997). Reeds and Burrin (2001), observing that intestinal cells are able of not only utilizing but also synthesizing the amino acid, hypothesized a “subtle” regulatory role of glutamine in modulating proliferation and differentiation rates.

Even if these studies as a whole suggest that the dose-efficacy of glutamine dietary supplementation has to be established considering the age of treated animals and the possible targeted intestinal tract, three aspects are to be underlined in considering this dietary supplement as potentially favourable: i) glutamine affects the structure of the piglet small intestine, above all jejunum and ileum; ii) the observed structural effects are detected in the villi height and crypt depth, values which appear enhanced, and thus the piglet intestinal mucosa is potentially able to restore the mucosal thinning that occurs at weaning; iii) the observed structural changes detected in comparison with non-treated animals may support, suggesting possible mechanisms of action, glutamine dietary supplementation in humans when pathologies (above all, chronic pathologies) develop in them linked to a loss of

intestinal mucosa (Alpers, 2000; Zhou et al., 2001).

Glutamate

Glutamate is an important constituent of dietary proteins and can be formed, with ammonia, from glutamine via glutaminase. Reeds et al. (1997) strongly suggest that glutamate is a major metabolic substrate for the intestinal epithelial cells. Using isotopic tracers in infant pigs, these authors found that labelled enteral glutamine was almost completely (95%) metabolized during its absorption through the intestinal epithelial layer. In a previous study conducted upon fed pigs (Reeds et al., 1994), enterally administered glutamate was demonstrated to be a preferential substrate for small bowel metabolism, and approximately 50% of it was metabolised to CO₂, a percentage higher than glucose in this model. Reeds et al. (2000) also showed that dietary glutamate appeared to be a specific precursor for the biosynthesis of glutathione, arginine and proline by the small intestinal mucosa. Fan et al. (2004) observed in neonate pigs that enterocytes utilize glutamate, delineating in addition a progression in their efficiency along the crypt-villus axis. Pigs fed a low-protein diet demonstrated suppressed oxidation of glutamate (and leucine) compared to controls fed a basal diet, while the percentage of glucose oxidized for energy obviously increased (Van der Schoor et al., 2001).

Taken together, these studies indicate that diet-derived glutamate plays an important role in intestinal physiology and metabolism at epithelial cell level. However, surprisingly little knowledge exists on the possible effects of glutamate upon the gut structure and on the possible roles of glutamate dietary

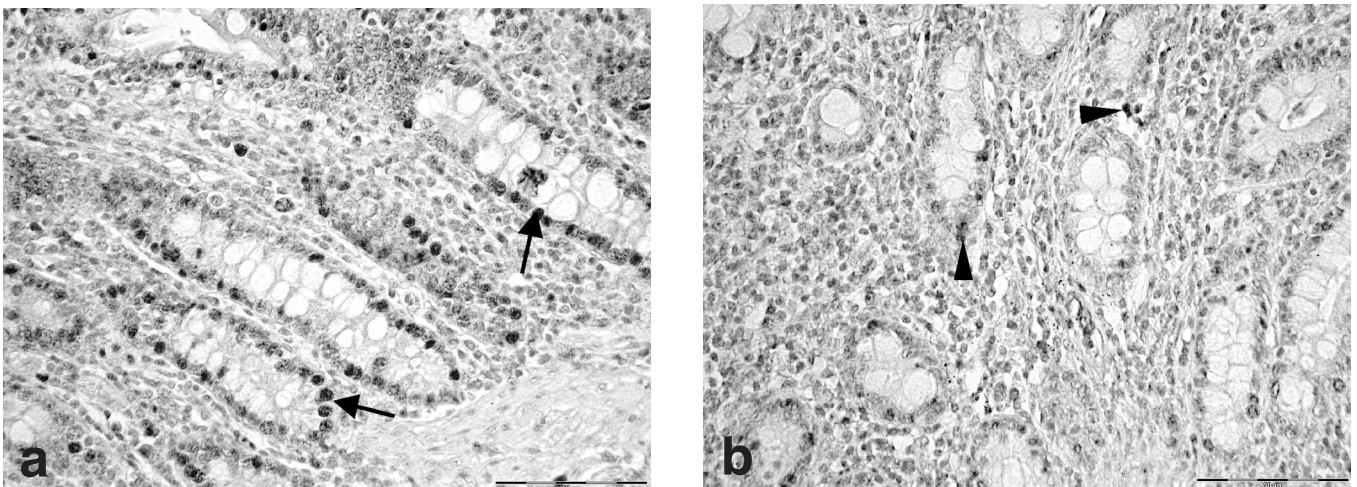


Fig. 1. **a.** Dietary Glutamine supplemented pigs: proliferating epithelial cells evident in ileum intestinal crypts (arrows). **b.** Control pigs: proliferating epithelial cells are less numerous in ileum intestinal crypts if compared with dietary glutamine administration (arrowheads). Scale bars: 50 μ m.

supplementation in models of gut inflammation, injury, or adaptation during a reparative process. Additional studies on the efficacy of dietary (or intravenous) glutamate as a trophic and cytoprotective nutrient are necessary.

Arginine

Arginine synthesis occurs primarily in the piglet small intestine (Stoll et al., 1998; Wu et al., 2004), and it is reputed to be conditionally indispensable in the neonate (Wilkinson et al., 2004), and for promoting intestinal repair (Rhoads et al., 2004). The net synthesis of arginine possibly needs proline as a primary dietary precursor, glutamate and glutamine not being fundamental in this metabolic pathway, above all in the neonate (Murphy et al., 1996; Wu, 1998; Wilkinson et al., 2004). Indeed, in an experiment employing arginine deficiency-induced hyperammonemia as the primary outcome, Brunton et al. (1999) demonstrated that arginine and proline are co-indispensable in intragastrically fed piglets. In that study, the authors concluded that the neonatal piglet couldn't synthesize sufficient quantities of arginine to maintain the urea cycle, as well as sufficient levels of proline to maintain plasma concentrations. The same Authors (Brunton et al., 1999) found that arginine synthesis from proline is dependent on gut metabolism, which is diminished as a result of the gut atrophy during parenteral feeding. In another study, Bertolo et al. (2000) demonstrated that the free metabolic pools of arginine, ornithine, citrulline, proline, glutamate, and glutamine were dramatically altered when identical diets were fed to piglets via the stomach, central venous or portal venous routes. In particular, they observed dramatic changes in the ornithine pools in liver, small intestinal mucosa, kidney, and plasma. They suggested that gut atrophy due to parenteral feeding caused lower nitrogen retention because of lowered conditionally indispensable amino acid (i.e., arginine and proline) synthesis by the atrophied gut (Bertolo et al., 1999). In an *in vitro* study, Rhoads et al. (2004) have recently hypothesized that arginine acts in promoting intestinal cell migration, in that it is an NO (nitric oxide) donator, a result which in turn underlines the importance of the potential link between arginine metabolism and NO synthesis. In severe septic conditions recreated in the swine animal model challenged with *E. coli* endotoxin, Bruins et al. (2002) have observed in the alimentary canal (and several other organs) an increased production of protective NO linked with an intravenous arginine supplementation.

In situations in which gut metabolism is compromised, such as during parenteral nutrition or severe gastrointestinal diseases (Bertolo et al., 2003), arginine is indispensable because its biosynthesis is negligible, and so this is a case (within a conspicuous number), in which the swine is to be considered an animal model for tentatively resolving intestinal human

pathologies, and data referring to it are potentially useful for humans.

Nucleotides

The terms "semi-essential" or "conditionally essential" have been used to describe the role of nucleotides in human nutrition. These nutrients may become essential when the endogenous supply is insufficient for the gut displaying its normal functions, even if their absence from the diet does not lead to a classic clinical deficiency syndrome. Conditions under which these nutrients may become essential include certain disease states in which a loss of gastrointestinal mass occurs, and periods of limited nutrient intake or rapid growth (such as the weaning period in food animal species). Under these conditions, intake of such nutrients as dietary integrators spares the organism the cost of a *de novo* synthesis of them, and may bring tissue metabolic levels to full working conditions (Uauy, 1989; Carver, 1999; Arnaud et al., 2003).

Nucleotides and their related metabolic products play key roles in many biological processes. They serve as nucleic acid precursors, physiologic mediators, constituents of coenzymes, and sources of cellular energy via respiratory pathways. The production of energy through the mitochondrial system of oxidation and reduction is severely impaired during the chronic diarrhoea statuses of early infancy (Arnaud et al., 2003), and this is a further occurrence in which a *de novo* nucleotide synthesis may be metabolically costly, and nucleotides can be obtained more efficiently from the diet or through the nucleotide salvage pathway. Although most dietary nucleotides are enzymatically metabolised and their final products excreted, up to 5% are incorporated into tissues, particularly during periods of rapid growth and limited food intake (Uauy, 1989; Carver, 1999).

A wide range of nucleotide concentrations, from 30 to > 70 mg/L, has been reported in human milk. Nucleotide-supplemented formulas of reconstituted milk generally contain between 20 and 70 mg/L (Carver, 1999), whereas concentrations of nucleotides in un-supplemented cow milk-based formulas are lower than those in human milk. Data suggest that dietary nucleotides play a role in the growth and differentiation of the gastrointestinal tract in neonatal ages. The intestinal tissues of animals fed nucleotide-supplemented diets have higher quantities of mucosal protein and DNA, higher villus height and disaccharidase activities, and better recovery after intestinal injury than do those of animals fed nucleotide-free diets (Uauy, 1989; Walker, 1996; Carver, 1999). Domeneghini et al. (2004) have recently found that piglets fed nucleotides revealed higher villus height and crypts depth than non supplemented animals, as well as higher numbers of mucosal macrophages and intra-epithelial lymphocytes. Very limited information is available about the young pig's needs for nucleotides, but because of the

effectiveness of dietary nucleotides in improving intestinal structure in its defensive aspects related to the maintaining of local health conditions and in the development of the immune system in other species, it may be speculated that nucleotides are needed by young pigs and human infants during periods of stress and infectious challenges.

Probiotics

Probiotics are non-pathological microorganisms (or components of bacteria), which, entering the alimentary canal with the diet and being able to reside in it during a limited period, are considered able to act as natural bio-regulators (Salminen et al., 1998a; Isolauri et al., 2002; Fig. 2). Commensals also of the mammalian digestive tract and their secretory products are to be considered within the large probiotic group. They are generally reputed to help in maintaining the balance of the digestive tract ecosystem by a variety of mechanisms (not yet, at present, fully clarified), and preventing the colonisation of the digestive tract by pathogenic bacteria possibly via a competitive exclusion mechanism (Vandernbergh, 1993). Recently, one of their roles in the modulation of inflammatory products (cytokines) secretion by stimulated intestinal epithelia has been demonstrated in an *in vitro* study (Bai et al., 2004).

In the fields of animal science and veterinary medicine, probiotics (above all lactic acid-producing bacteria, bifidobacteria, *Bacillus* spp. bacteria, and yeast) may be effectively used especially in improving digestive processes in both young and adult life stages, in stimulating growth and other productive parameters, as well as in preventing digestive tract diseases above all in young farm animals (Casey et al., 2004). In human therapy also they may be considered a useful modality (in a possible co-administration of antibiotics) for treating intestinal disorders, both acute and chronic (Vanderhoof, 2001; Steidler, 2003).

The data concerning the efficacy of probiotics in practice are often contradictory. By dietary administration of probiotic lactobacilli to pigs, many authors have reported a stimulatory effect upon growth

(Baird, 1977; Hale and Newton, 1979; Pollmann et al., 1980; Nousianinen and Settälä, 1993). The effect of lactobacilli and bifidobacteria aimed at contrasting intestinal infections with varying forms of diarrhoea in pigs was confirmed by several reports (Hale and Newton, 1979; Kimura et al., 1983; Maeng et al., 1989; Bomba et al., 1998; Depta et al., 1998). However, other authors (De Cupere et al., 1992; Bekaert et al., 1996) have not described this effect, surely on the basis of different species of microorganisms responsible for intestinal infections, as well as the intestinal tract primarily interested as the source of infection.

The variable degrees of efficacy of probiotics under different conditions may be due to the probiotic preparation itself or may be caused by other factors, comprising the nutritional and sanitary status of the treated animals (Mao et al., 1996), and the effects of age, stress, and genetic differences. Variability of the data referring to the used probiotic (or mixture of different probiotics) may be due to: different survival rates of species and strains, different preferred adhesion to the small rather than large intestine, stability of the species/strains within the alimentary canal, and their survival in the gastric acid compartment and to the interaction with biliary acids, the use of a non-specific strain relative to the host, doses and frequency of administration not fully appropriate, interactions with chemotherapeutics (Casey et al., 2004; Ohashi et al., 2004). Research experience points to the fact that probiotics are most effective in animals during microflora development (young ages) or when microflora stability is impaired (Stavric and Kornegay, 1995).

A probiotic species/strain, in addition to being non-pathogenic and able to tolerate the conditions of the digestive tract, should adhere with high numbers of individuals to the digestive tract mucosa, even if for a limited period, sufficient to stimulate local defensive processes and improve the intestine's barrier function. As a consequence, competitive exclusion of pathogens and harmful antigens will occur. In addition, probiotic microorganisms should be able to maintain high viability during industrial processing, and after lyophilisation and storage, to re-vitalise quickly in the digestive tract, and to produce inhibitory substances against pathogens. Some of the above-mentioned criteria for the selection of microorganisms for probiotic purposes can be tested *in vitro*, but most of them must be verified *in vivo*. Actually, some properties of microorganisms observed under laboratory conditions have not been confirmed in trials with animals (Chateau et al., 1993; Bomba et al., 1998).

The efficacy of probiotics may be enhanced by the following methods: 1) selection of species (host)-specific strains of microorganisms (Isolauri et al., 2002) or of intestinal disorder-specific strains (Shanahan, 2002); 2) genetic engineering, aimed at possibly enhancing the effectiveness of existing species/strains and introducing into the genome of probiotics some

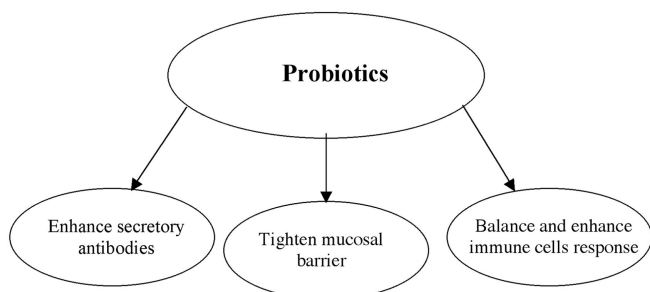


Fig. 2. Reputed functional roles of probiotics upon intestinal microhabitat

special elements of regulatory systems or enzymes derived from both foreign and human sources, in such a way adding special properties (Steidler, 2003). Even if attention is to be paid to possible dangers linked to the release of genetically modified microorganisms, a promising aspect of engineered probiotics is to induce in them the secretion of biologically active cytokines (Steidler and Neirynek, 2003; Steidler et al., 2003), aimed at reducing the side effects of traditional therapies during chronic inflammatory bowel diseases; 3) combination of different probiotic species/strains, which show different sites of adhesion in the alimentary canal and action mechanisms; 4) combination of probiotics and synergistically acting components (synbiotic: see below); 5) inactivation of some strains, with the aim of obtaining a major degree of safety, a longer shelf life, a minor degree of interactions with other components of the meal. Even if, generally, viable probiotics are to be considered largely effective in stimulating defensive responses, non-viable probiotics may be more effective than viable ones when the inactivation of an intestinal toxin is to be obtained (Salminen et al., 1998b).

In order to evaluate and possibly enhance the efficacy of a probiotic, it is necessary to obtain important knowledge about the possible mechanisms mediating the effects in the digestive tract (Stavric and Kornegay, 1995), an argument which is not yet fully clarified (Sakata et al., 2003). Actually, the possible anti-bacterial effect of a probiotic microorganism or its beneficial effects on the entire host macroorganism may be mediated by one or multiple mechanisms that may be expressed at different degrees of intensity.

Sakata et al. (2003) have recently described a potential mechanism linked to the increased production of short-chain fatty acids (SCFAs) (modulators of several gut functions) in the caecum of pigs dietary supplemented with probiotic bacteria, which in turn accelerate the breakdown of carbohydrates. Other studies indicate that in pigs, the intestinal morphology (and, possibly, function) of the epithelium may be modified by probiotics. Significant longer villi and deeper crypts were measured in the ileum of piglets receiving diets supplemented with the yeast *Saccharomyces cerevisiae* ssp. *bouardii* (Di Giancamillo et al., 2003) and *Pediococcus acidilactici* (Di Giancamillo et al., 2004), respectively, and this without any sign of hyperplastic or other pathological aspects. These modifications may be considered beneficial because they enable the young pigs to both respond to the growing necessities of their gastrointestinal apparatus and confront possible bacterial or viral challenges. Other authors found longer intestinal villi in the jejunum of pig supplemented with *Bacillus cereus toyoi* or *Saccharomyces bouardii*, respectively (Gorke and Liebler-Tenorio, 2001). The number of goblet cells and the type of secreted mucins appeared in part modified in the large bowel of pigs dietary supplemented with *Bacillus cereus toyoi* or *Saccharomyces bouardii* (Baum et al., 2002).

In vitro studies indicate that some probiotics (*Lactobacillus plantarum* 299v and *L. rhamnosus* GG) have the ability to inhibit adherence of attaching and effacing of pathogen *E. coli* HT 29 to intestinal epithelial cells by increasing expression of the intestinal MUC2 and MUC3 mucins (Mack et al., 1999).

Intestinal mucosa from pigs, which were adapted to diets containing *Bacillus cereus* or *S. bouardii*, had an increased barrier function and modified nutrient transport kinetics for glucose and aminoacids (Baum et al., 2002). Rats which were pre-treated with *Lactobacillus plantarum* 299v were protected against the *E. coli*-induced increase in intestinal permeability (Mangell et al., 2002). The processes which mediate these responses on the cellular and/or tissue level, are unknown, and research on the problems is in progress in several laboratories.

Even if the preventive and, perhaps, therapeutic uses of probiotics are promising tools for the treatment and prevention of gastrointestinal (and perhaps, extra-intestinal) diseases, their utilization for both food animal species and humans deserves some precautions, linked to their established interactions with immune cells (Gills, 1998) and the possible occurrence of allergic diseases (Paganelli et al., 2002).

Prebiotics

The prebiotic concept has been developed considering that both selective growths of resident intestinal bacteria is recommended, with the aim of synthesizing vitamins and stimulating local immunity, and viability of administered useful microorganisms in food products and during transit through the host gastrointestinal tract is variable and not always predictable. Most commonly, prebiotics are not absorbable carbohydrate substrates or food ingredients that are non-digestible (except through bacterial activity). They selectively stimulate the growth of both resident bacterial species and dietary administered probiotics, which in turn can improve the host health (Gibson and Roberfroid, 1995; Isolauri et al., 2002; Teitelbaum and Walker, 2002). When probiotics and prebiotics are administered together, we can define the mixture as synbiotic (Isolauri et al., 2002). It seems that a number of suitable components (oligosaccharides, phyto-components, nutrients and growth factors, proteins, polyunsaturated fatty acids, organic acids and bacterial metabolites) may be used in pigs to potentiate the effect of probiotics (Pollmann et al., 1980; Gálfi and Bokori, 1990; Gibson and Roberfroid, 1995; Yadava et al., 1995; Breves et al., 2001; Bomba et al., 2002; Smiricky-Tjardes et al., 2003; Konstantinov et al., 2004).

The prebiotic approach advocates the administration of nonviable entities, and therefore overcomes the already mentioned problems of probiotic microorganism survival in the upper gastrointestinal tract. The possible results of this interaction substantially fall within an improved resistance of the host to gut pathogens. In fact,

the prebiotic-derived stimulation of lactic acid bacteria of the human gastrointestinal tract is thought to play a significant role in an improved colonization resistance towards pathogens (Gibson et al., 1997; Glenn, 1999). Similarly, increased numbers of bifidobacteria in the gut of breast-fed infants as a consequence of prebiotic administration to their mothers may contribute to the improved competitive exclusion of pathogens seen in this group compared with those infants who have been formula-fed (Gibson et al., 1997).

Prebiotics include fructooligosaccharides (FOS), which, when dietary administered, reach the large intestine and are fermented into short-chain fatty acids (SCFAs), lactate, and carbon dioxide. As the major energy source for the epithelial cells of the large intestine, n-butyrate stimulates the proliferation of cells as well as mineral and water absorption from the lumen. Tsukahara et al. (2003) found that the crypt depth was higher in the large intestine of the FOS-fed piglets in comparison with controls. Also Van Nevel et al. (2003) found that small intestinal villi length was increased in piglets fed non-digestible oligosaccharides if compared with control animals. Some of these effects may possibly be linked to the inhibition of enterocyte apoptosis, as Claus et al. (2003) have recently hypothesized in the pig colon. FOS stimulate higher rates of colonocyte proliferation than cellulose and other non-digestible oligosaccharides do in pigs, without increasing the measured total amount of mucosa, as well as enhance the effects of probiotic bacteria in the large intestine (Bomba et al., 2002). They can in addition alter the composition of the human gut resident microflora, by a specific fermentation pathway, towards a community predominated by bifidobacteria (Gibson, 1999). Human milk also contains FOS and other complex oligosaccharides (Taitelbaum and Walker, 2002), possibly aimed at stabilizing the resident gut microflora of the infant.

Despite the established effects of prebiotics in modifying gut microflora, they do not always display the same health promoting effects of probiotics (Branner et al., 2004), possibly due to their effects being limited to the time during which they are consumed.

Conclusion

The phasing out of antibiotics as growth promoters from the animal industry has renewed the interest for alternative nutritional strategies, for both increasing the farm animal's performances in the absence of antimicrobial growth promoters, and protecting them against numerous pathologies and physio-pathological disturbances, which frequently occur in farm reality. In this context, the use of feed additives, such as those considered in this review, may be of potentially very high interest in rearing food animal species, especially swine.

The swine is an omnivore like humans, and in the field of nutrition also, as in other scientific fields, the

swine is universally considered an indispensable animal model for studying and resolving human concerns. Even if great attention is to be paid in transferring results obtained from experimental animal studies to human use (as well as from in vitro studies), the use of the swine animal model may help in describing or hypothesizing some rationales and mechanisms of action of dietary supplementations, and this in turn may adequately support different preventive and/or therapeutic approaches in the use of gut-trophic additives.

The results of the reviewed studies evidence that micro-anatomical analyses, above all those quantitative ones aimed at objectively measuring gut structural details changed in relation to dietary supplements, are potentially useful in assessing the real utility of a gut-trophic factor for a certain species, obviously not alone but in conjunction with other scientific research fields. This in turn appears very promising in view of a multi-disciplinary approach for the validation of these special dietary additives for both animal and human medicine, in which a merely practical application is often short-sighted and auto-limiting.

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