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Impact of bioactive substances on the gastrointestinal tract and performance of weaned piglets: a review*

J. P. Lallès[1†], P. Bosi[2], P. Janczyk[3], S. J. Koopmans[4] and D. Torrallardona[5]

[1]*INRA UMR 1079, Rearing Systems, Animal and Human Nutrition, Domaine de la Prise, 35590 Saint-Gilles, France;* [2]*Animal Nutrition, DIPROVAL, University of Bologna, via Rosselli 107, 42100 Reggio Emilia, Italy;* [3]*Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany;* [4]*Animal Sciences Group of Wageningen UR, Animal Production Division, PO Box 65, 8200 AB Lelystad, The Netherlands;* [5]*IRTA, Ctra. Reus-El Morell, km. 3.8, E-43120 Constantí (Tarragona), Spain*

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The EU ban on in-feed antibiotics has stimulated research on weaning diets as a way of reducing post-weaning gut disorders and growth check in pigs. Many bioactive components have been investigated but only few have shown to be effective. Amongst these, organic acids (OA) have been shown to exert a bactericidal action mediated by non-dissociated OA, by lowering gastric pH, increasing gut and pancreas enzyme secretion and improving gut wall morphology. It has been postulated that they may also enhance non-specific immune responses and improve disease resistance. In contrast, relatively little attention has been paid to the impact of OA on the stomach but recent data show they can differently affect gastric histology, acid secretion and gastric emptying. Butyrate and precursors of butyric acid have received special attention and although promising results have been obtained, their effects are dependent upon the dose, treatment duration, initial age of piglets, gastrointestinal site and other factors. The amino acids (AA) like glutamine, tryptophan and arginine are supportive in improving digestion, absorption and retention of nutrients by affecting tissue anabolism, stress and (or) immunity. Glutamine, cysteine and threonine are important for maintaining mucin and permeability of intestinal barrier function. Spray-dried plasma (SDP) positively affects gut morphology, inflammation and reduces acquired specific immune responses via specific and a-specific influences of immunoglobulins and other bioactive components. Effects are more pronounced in early-weaned piglets and under poorer health conditions. Little interaction between plasma protein and antibiotics has been found, suggesting distinct modes of action and additive effects. Bovine colostrum may act more or less similarly to SDP. The composition of essential oils is highly variable, depending on environmental and climatic conditions and distillation methods. These oils differ widely in their antimicrobial activity in vitro *and some components of weaning diets may decrease their activity. Results in young pigs are highly variable depending upon the product and doses used. These studies suggest that relatively high concentrations of essential oils are needed for beneficial effects to be observed and it has been assumed that these plant extracts mimic most of the effects of antibiotics active on gut physiology, microbiology and immunology. Often, bioactive substances protective to the gut also stimulate feed intake and growth performance. New insights on the effects of selected OA and AA, protein sources (especially SDP, bovine colostrum) and plant extracts with anti-bacterial activities on the gut are reported in this review.*

Keywords: bioactive substance, gastrointestinal tract, nutrition, pig, weaning

Introduction

Weaning is a critical period in a piglet's life and is accompanied by nutritional, social and environmental stress. At weaning, the diet is changed from a highly digestible and liquid diet (the milk) to a solid, more complex and less-digestible diet, and post-weaning gut disorders cause important economic losses in the pig industry. A transient anorexia as usually observed after weaning leads to gut dysfunction, increased sensitivity to enteric infections and diarrhoea. The most consistent patho-physiological changes effect the anatomy and function of the small intestine (Pluske *et al.*, 1997), and these include a 20–30% reduction in mucosal weight associated with villous atrophy (Lallès *et al.*, 2004).

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† E-mail: Jean-Paul.Lalles@rennes.inra.fr

Further recent studies have shown that intestinal barrier function is compromised, resulting in increased secretion of electrolytes and water and increased permeability to potentially toxic substances (reviews by Vente-Spreeuwenberg and Beynen, 2003; Lallès *et al*., 2004). In addition to alterations in gut physiology, weaning causes disturbances in the homeostasis of the gut microbiota and delays the anatomical and functional development of the mucosal immune system (review by Lallès *et al*., 2007).

In-feed antibiotic growth promoters (AGP) have been long considered as acting essentially on the gut microbiota by decreasing the potentially harmful effects of pathogenic bacteria (review by Dibner and Richards, 2005). Such AGP were more effective in promoting growth in young animals such as in piglets after weaning, and when hygiene conditions are poor (review by Anderson *et al*., 1999). Complementary mechanisms of action centred on the gut microbiota have been described together with the consequences of supplemental AGP on improvements of gut homoeostasis, feed component digestibility, feed efficiency and growth performance. However, AGP may also act directly on the host and one recent hypothesis would suggest that AGP would display non-antibiotic anti-inflammatory properties (Niewold, 2007). This in turn would explain a lower protein catabolism related to a reduced level of inflammatory cytokines and, therefore, a better growth performance.

The use of in-feed AGP has been banned in the EU since 1 January 2006 and many attempts at alternative approaches have been applied to replace them. However, the success rate is low due to the complexity of the aetiology and pathogenesis of gastrointestinal tract (GIT) disorders on the one hand, and to the frequent lack of knowledge on the mechanisms of action and effective doses of alternative substances on the other hand. This has led to intensive research over Europe to increase our understanding on the mechanisms of action of known substances, used alone or in combination, and for exploring the possibilities offered by new substances. Such potential bioactive feed additives include nutrients (e.g. particular amino acids (AA)), mineral and organic acids (OA), minerals, growth factors, prebiotics and probiotics, and finally phytogenic compounds. All these substances have in common their potential for protecting the gut, albeit through very different ways, including mucosal growth, intestinal barrier function strengthening, anti-oxidant and anti-inflammatory capacities, and finally anti-bacterial properties.

The aim of this review is to gather recent findings on a limited number of categories of AGP alternatives, including OA (and sodium butyrate (SB)), specific AA (Gln, Trp, Arg, Cys, Thr), animal proteins (e.g. spray-dried plasma (SDP) and bovine colostrum) and plant extracts with known anti-bacterial activities *in vitro*. These substances have been studied more extensively within the frame of two EU projects (Healthypigut, 2000; Feed for Pig Health, 2003) and some of these substances offer a real alternative to AGP for protecting the gut and maintaining animal performance, whilst others display interesting protective properties *in vitro* or *ex vivo* but there are limited data *in vivo* and/or evidence of their protective efficacy.

Organic acids

The effects and modes of action of OA have been reviewed recently (Partanen, 2001; Roselli *et al*., 2005; Bannink *et al*., 2006; Mroz *et al*., 2006). Some highlights from these reviews involve the *in vivo* trials with acidified diets in weaned piglets. From formic, fumaric and citric acids and potassium diformate, sufficient data could be gathered for meta-analysis (Partanen, 2001). From this analysis it was concluded that formic acid and potassium diformate were most potent in increasing feed consumption and average daily gain (ADG). Increased feed intake is thought to reflect improved health after weaning and is crucial for high growth. Another highlight from these reviews includes the bactericidal potency of OA. In a porcine intestinal organ culture model, it was established that coliform bacteria were most effectively killed by benzoic > fumaric > lactic > butyric > formic > propionic acid. With respect to *Salmonella typhimurium* the order was benzoic > sorbic > lactic > propionic > formic > acetic acid. Overall, benzoic acid is most effective, lactic acid is intermediate and both formic and propionic acids are least effective at killing both coliforms and *S. typhimurium*.

The stomach contents play an important role in limiting the entry of bacteria into the intestine. The inclusion of a combination of 1% lactic acid and 1% formic acid in the diet reduced gastric pH and concentration of lactic acid bacteria and enterobacteria (Hansen *et al*., 2007). Lactic acid bacteria were also reduced by 0.5% benzoic acid (Guggenbuhl *et al*., 2007) and by the addition of 200 mEq/kg of a 1 : 1 formic : fumaric acid mixture, but not by a 1 : 1 formic : lactic acid mixture (Franco *et al*., 2005). Benzoic acid at 0.5% or 1.0% reduced the number of total aerobic, total anaerobic, lactic acid forming and Gram-negative bacteria in the stomach (Kluge *et al*., 2006). In another study benzoic acid at 0.5% improved growth and feed efficiency, but had no effect on reducing the number of culturable Lactobacilli, Enterococci, *Escherichia coli* and *Clostridium perfringens* from the ileum and caecum as measured with traditional culture methods (Torrallardona *et al*., 2007a). However, these authors did observe a marked increase in the biodiversity of the ileal microbiota, which suggests a more stable microbial ecosystem (Zoetendal *et al*., 2004). Potassium diformate has also been shown to reduce *Salmonella* shedding in *Salmonella*-challenged piglets (Papenbrock *et al*., 2005). Finally, inclusion of an OA mixture with a lipid matrix was found to increase the concentration of OA in the distal GIT and reduced the number of coliforms in the caudal jejunum and in the caecum (Piva *et al*., 2007).

Very little attention has been paid to the impact of OA on the stomach. Gastric mucosa absorbs and transports volatile fatty acids (VFA) at substantial rates and OA are rapidly recovered from the blood after a single feed (Hanzlik *et al*., 2005). Concentrations of OA decline rapidly between

the stomach and the duodenum (e.g. formic acid; Canibe *et al*., 2005), suggesting that pig proximal GIT is an important site of OA absorption. VFA are transported by the monocarboxylate transporters (MCT). The transporter MCT1 is most widely distributed in the GIT and has a specific role in lactate and butyrate transport. The presence of MCT1 has been detected by immuno-staining on the surface of adult mouse gastric surface epithelium (Nakai *et al*., 2006). The distribution of MCT1 was studied in the small intestine and colon of pigs (Ritzhaupt *et al*., 1998a and 1998b; Sepponen *et al*., 2007), and its presence was enhanced by the luminal production of butyrate after bacteria fermentation. The addition of different OA to the diet can differently affect the morphology and function of the stomach of weaned pigs. In the fundic mucosa, Ca-formate reduced the number of HCl-secreting parietal cells and H^+/K^+ ATPase gene expression (Bosi *et al*., 2006). Conversely, it increased numbers of cells secreting somatostatin, which is suppressive to HCl secretion. Fat-protected formic acid was also tested in piglet feeding, to explore the effect of a delayed release of this OA on gut barrier function. Interestingly, this product did not cause the gastric changes observed with free OA, confirming its direct impact on oxyntic mucosa (Bosi *et al*., 2006).

The effect of OA supplementation on gastric emptying and on the rate of digesta passage has not been studied in pigs. In humans, oral intake of propionate reduced the gastric emptying rate (Darwiche *et al*., 2001), and this could explain the increased weight of gastric content and dry matter (DM) percentage after supplementation with formic acid to early-weaned pigs (Manzanilla *et al*., 2004). An increased stasis of feed in the stomach can permit a deeper action of acid secretion and this can be seen particularly favourable in a dirty environment. Conversely, it can also reduce feed intake. Addition of OA can have negative effect (Eisemann and van Heugten, 2007), no effect (Partanen, 2001; Ettle *et al*., 2004) or favourable effect (Partanen, 2001) on feed intake in piglets. This could be related to the type or dose of OA used, the differences in taste and smell, or from differences in local sensing of OAs. More research attention should be placed on the role of stomach in the action of OA in the GIT of young pigs.

The dietary effect of OA has also been studied in weaning pigs orally infected with pathogenic *Escherichia coli* K88 (Table 1). Dietary supplementation with free Ca-formate, but not with fat-protected formate, improved post-challenge growth, feed intake, gain to feed ratio and reduced the faecal score, days of diarrhoea and faecal excretion of total (but not K88-specific) *E. coli*, and increased villous height in the small intestine (Bosi *et al*., 2007). The effects were seen independent of the individual piglet phenotypes for intestinal adhesion of *E. coli* K88. After a challenge with *E. coli* K99, Ca-formate improved neither growth performance nor total *E. coli* count in ileum, but jejunal villous height was reduced (Torrallardona *et al*., 2007b). Earlier work with pathogenic challenges indicated that 2% fumaric acid tended to improve average daily gain (ADG) over the first 2 weeks post-weaning and to reduce scours, faecal shedding of *E. coli* K88 and mortality (Owusu-Asiedu *et al*., 2003b). In a study with *Lawsonia intracellularis* challenge 2.4% lactic acid, but not 1.8% fumaric acid was able to reduce intestinal lesions induced by this pathogen (Boesen *et al*., 2004).

The case of sodium butyrate

As a result of their well-known beneficial effects on colonic mucosa, butyrate and butyrate precursors have been the focus of special attention (review by Hamer *et al*., 2008). However, little is known of the effect of oral butyrate on the small intestine.

SB is more frequently used in pig feeding due to its less-strong odour than the acid. It is presumed that the effect inside the gut is not different, because SB dissociates. In experiments conducted by Manzanilla *et al*. (2006) and Castillo *et al*. (2006), SB was included in the starter diet at 3 g/kg and fed for 14 days to piglets weaned at 18–22 days of age. SB supplementation improved feed gain ratio in week 2 post-weaning and over the 14-day post-weaning period. SB was detected only in the stomach, suggesting quick gastric and (or) duodenal absorption or catabolism (Gálfi and Bakori, 1990; Manzanilla *et al*., 2006). SB increased gastric DM percentage (Manzanilla *et al*., 2006). SB supplementation also induced large changes in both the biodiversity of the microbial ecosystem and species composition of the bacterial community in the jejunum (Castillo *et al*., 2006). Total microbial activity in digesta was lower in the caecum and distal colon when SB was present in the diet. Manzanilla *et al*. (2006) suggested that SB supplementation may have contributed to stabilise the gastrointestinal ecosystem while depressing amilolytic bacteria, thus improving the health status of the pigs and the efficiency of the use of nutrients for growth.

A range of doses of SB (0, 1, 2 and 4 g/kg) was tested for 6 weeks in another study of piglets weaned at 28 days (Biagi *et al*., 2007). SB had effects on neither growth performance (despite a numerical improvement with the highest dose) nor intestinal mucosa morphology. The only reported changes were increased caecal pH and isobutyric acid concentration with a tendency for increased ammonia concentration. This suggested that oral SB stimulated protein catabolism in the caecum of pigs. However, the underlying mechanisms are unresolved thus far. The authors considered the bacterial hypothesis as unlikely because the counts of clostridia, enterobacteria and lactic acid bacteria along the intestines were unaffected by SB (Biagi *et al*., 2007).

The period of oral SB administration has also recently been investigated by another group (Le Gall *et al*., 2007). SB (0.3 g/kg of milk or feed intake) was provided to piglets either during the suckling period (day 4 to day 28) or after weaning (day 28 to day 39–40) or both before and after weaning (day 4 to day 39–40). SB stimulated growth rate before weaning and over the entire period of observation, whilst feed intake after weaning was enhanced when SB

Table 1 *Average daily feed intake (ADFI), weight gain (average daily gain (ADG)) and feed conversion ratio (FCR) obtained in different studies on weaning piglets experimentally challenged with different* Escherichia coli *strains when spray-dried plasma (SDP) or other substances were fed to the animals*

Supplement, its amount	ADFI (kg)	ADG (kg)	FCR	*E. coli* strain, dose, day[a]	Weaned (days of age)	Mean BW at weaning (kg)	Duration of study (days)	Reference
Control	0.225^{xy}	0.150^{x}	1.56	K99, 5×10^7 colony forming unit (CFU), day 0	21	6.2	14	Torrallardona *et al.* (2007b)
Antibiotic[b]	0.236^{xy}	0.173^{xy}	1.37					
Ca formate, 18 g/kg	0.202^{x}	0.141^{x}	1.47					
SDAP[c], 60 g/kg	0.301^{y}	0.223^{y}	1.35					
Control	0.192	0.112	1.78	K99, 5×10^7 CFU, day 5	21	7.4	10	Torrallardona *et al.* (2007b)
Antibiotic[b]	0.196	0.128	1.52					
Ca formate, 18 g/kg	0.172	0.106	1.65					
SDAP[c], 60 g/kg	0.230	0.142	2.01					
Control	0.263	0.182^{x}	1.47^{x}	K99, 1×10^7 CFU, day 5	21	7.3	14	Conde (2005)
Antibiotic[b]	0.312	0.252^{y}	1.26^{y}					
SDAP[c], 60 g/kg	0.312	0.245^{y}	1.28^{y}					
SDAP[d], 60 g/kg	0.275	0.199^{xy}	1.37^{xy}					
Control	0.175	0.128	1.81	K88, 1×10^{10} CFU, day 4	21	4.9	15	Bosi *et al.* (2004)
Antibiotic[e]	0.186^{f}	0.148	1.48					
SDP, 60 g/kg	0.197^{g}	0.164^{g}	1.30					
SDP, 60 g/kg + Antibiotic[e]	0.214^{fg}	0.186^{g}	1.30					
Control	0.114	0.085^{x}	1.35	K88, 6×10^{10} CFU, day 7	10	3.5	14	Owusu-Asiedu *et al.* (2003a)
IgY[h], 5 g/kg	0.157	0.123^{xy}	1.27					
SDPP[i], 50 g/kg	0.173	0.132^{y}	1.31					
SDPP[i], 100 g/kg	0.167	0.127^{xy}	1.31					
SDPP[i], 100 g/kg + IgY[h], 5 g/kg	0.174	0.130^{xy}	1.34					
Control	0.141	0.101	1.40	K88, 6×10^{10} CFU, day 7	10	3.8	14	Owusu-Asiedu *et al.* (2003b)
Antibiotic[j]	0.222	0.153	1.46					
IgY[h], 5 g/kg	0.208	0.151	1.38					
Zn oxide, 2880 mg/kg	0.215	0.159	1.35					
Fumaric acid, 20 mg/kg	0.212	0.155	1.36					
SDPP[i], 100 g/kg	0.213	0.157	1.36					
Control	0.227	0.101	2.33	K99, 5×10^7 CFU, day 0	24	7.1	14	Torrallardona *et al.* (2003)
Antibiotic[b]	0.298^{f}	0.214^{f}	1.41^{f}					
SDAP[c], 70 g/kg	0.268	0.193^{g}	1.39^{g}					
SDAP[c], 70 g/kg + Antibiotic[b]	0.286^{f}	0.230^{fg}	1.23^{fg}					
Control	0.226	0.101	2.25	K88, 1×10^{10} CFU, day 4	19	4.9	15	Bosi *et al.* (2001)

Table 1 *Continued*

Supplement, its amount	ADFI (kg)	ADG (kg)	FCR	*E. coli* strain, dose, day[a]	Weaned (days of age)	Mean BW at weaning (kg)	Duration of study (days)	Reference
SDAP[c], 250 g/kg	0.252	0.169	1.49					
SDAP[d], 250 g/kg	0.251	0.139	1.80					
SDPP[i], 250 g/kg	0.251	0.100	2.52					
Control	0.197	0.100[x]	1.96	K88, 1×10^{10} CFU, day 2	13	4.2	14	Bosi *et al.* (2001)
SDAP[c], 250 g/kg	0.215	0.140[y]	1.53					
SDAP[d], 250 g/kg	0.208	0.107[x]	1.96					
SDPP[i], 250 g/kg	0.208	0.118[x]	1.78					

[a]Day post-weaning at which the experimental infection was performed.
[b]Colistin 300 mg/kg diet.
[c]IgG-standardised and pelleted spray-dried plasma from animal (bovine and porcine) origin.
[d]Non-standardised spray-dried plasma from animal (bovine and porcine) origin in powder form.
[e]Colistin 250 mg/kg diet and amoxycycline 500 mg/kg diet.
[f]Statistically significant effect of antibiotic addition over control.
[g]Statistically significant effect of SDAP addition over control.
[h]Egg yolk antibodies from hens immunised against *E. coli* K88 and *E. coli* F18.
[i]Spray-dried plasma from porcine origin.
[j]Carbadox 55 mg/kg diet.
[x,y]Different superscripts mark significant difference between values in a column within one study.

was fed during the suckling period. Supplementing SB before and (or) after weaning increased the number of HCl-secreting parietal cells per gland observed at 7-day post-weaning (Mazzoni *et al.*, 2008). This effect was seen notwithstanding the parallel increase of somatostatin-secreting cells. The weights of the whole tissue and mucosa of the small intestine were reduced with SB feeding, with minor changes in villous-crypt architecture and digestive enzyme-specific activities (Le Gall *et al.*, 2007). These changes were very similar to previous findings in a study with plasma protein (Jiang *et al.*, 2000b) that showed suppression of both the inflammatory response and AA catabolism in the intestine. Whether SB alters intestinal inflammation and AA metabolism is presently unknown.

The protective effects of butyrate are well documented (review by Hamer *et al.*, 2008) and an alternative approach to delivering butyrate to the gut has been by providing the animal with butyric acid precursors, including tributyrin and lactitol (Piva *et al.*, 2002) or gluconic acid (Tsukahara *et al.*, 2002; Biagi *et al.*, 2006a and 2006b). Supplementing the piglets with tributyrin or lactitol reduced mortality and this protective effect was enhanced when both were fed together (Piva *et al.*, 2002). The combined supplementation increased growth performance and jejunal villous height while reducing histamine levels in jejunal and caecal tissues and caecal crypt depth. Tributyrin alone had adverse effects on growth performance. It did not influence jejunal villus height but it reduced drastically caecal crypt depth. Lactitol alone stimulated jejunal villous height and the level of lactic acid in the caecum while reducing caecal crypt depth. Gluconic acid tended to improve growth rate without changes in intestinal morphology (Biagi *et al.*, 2006a) and to support longer ileal crypts and shorter caecal crypts (Biagi *et al.*, 2006b).

Collectively, the results obtained with an oral supplementation of SB or with butyrogenic precursors indicate that the outcome may depend on various factors including dose, supplementation duration, site of observation, age of pigs at initiation of butyrate supplementation and probably other uncontrolled factors. As an example, an interaction between butyrate infused in the caecum of pigs and dietary inulin was recently demonstrated (Kien *et al.*, 2007). Butyrate alone stimulated epithelial cell proliferation along the intestines but dietary inulin tended to block this effect. The precise modes of action of butyrate in the small intestine are not yet fully understood.

Specific amino acids

Apart from their role as building blocks for peptides and proteins, several essential and non-essential AA are thought to have therapeutic effects on the GIT and on the whole organism (review by Kim *et al.*, 2007). Specific AA can promote health by improving (gut) tissue anabolism, by reducing the impact of stress and by modulating local immunology (Table 2). This could be beneficial when growth is inhibited by infection, inflammation and stress

Table 2 *Summary of the effector molecules and bioactivities of five bioactive amino acids (adapted from D'Mello, 2003)*

Amino acid as precursor	Effector molecule	Bioactivity and pig response
Arginine	Nitric oxide	Vasorelaxation, neurotransmission, gut motility
	Polyamines	Regulation of RNA synthesis, maintenance of membrane stability
Cysteine	Cysteine	Important for activity of proteins
	Glutathione	Immune response (T-cell activity and antibody response)
Glutamine	Purine and pyrimidine	Energic source in some tissues (mucosa)
Threonine	Threonine	Immune response (IgG antibodies)
	Glycoproteins	Mucin production
Tryptophan	Tryptophan	Immune response (acute phase proteins)
	Serotonin	Neurotransmission, appetite, coping with stress
	Kynurenine	Immune response including T-cell activity and free radical scavenger production
	Nicotinamide	B-complex vitamin

(Gruys *et al*., 1998). Biologically active AA can be added to the diet in case of a deficiency during disease and experimental results suggest that the profile of AA required for the immune system differs substantially from that for growth (Reeds *et al*., 1994). In addition, surplus administration of certain dietary AA has been shown to be necessary for inducing significant bioactivity for therapeutic purposes (Massey *et al*., 1998). From the literature, five AA, namely glutamine, tryptophan, arginine, cysteine and threonine, showed consistent bioactivity with respect to physiology, immunology, endocrinology and metabolism.

Glutamine (Wu *et al*., 1995) and glutamate (Reeds *et al*., 2000) are important fuels for intestinal epithelial cells. Notwithstanding the stresses of weaning, these AA were able to maintain the high turnover of the gut mucosa, as indicated by longer villi in pigs supplemented with glutamine (Ayonrinde *et al*., 1995; Liu *et al*., 2002; Lee *et al*., 2003; Domeneghini *et al*., 2004 and 2006) or glutamate (Liu *et al*., 2002). For glutamine, this was determined by a decrease in the apoptotic to mitotic mucosal cell ratio (Domeneghini *et al*., 2004 and 2006). The positive influence of glutamine on intestinal architecture and growth performance in healthy swine persists in swine at risk of colibacillosis, as shown in *E. coli* K88-challenged piglets (Yi *et al*., 2005). Interestingly, the small intestine utilises glutamine to synthesise citrulline and after rotavirus infection, blood plasma citrulline values were reduced, while glutamine increased (Rhoads *et al*., 2007). Thus utilisation of glutamine in the intestine was reduced for the compromised number of villous cells. Systemic glutamine may also influence the gut since its parenteral administration decreased intestinal permeability and endotoxin levels in burns patients (Zhou *et al*., 2004).

Glutamine is rapidly utilised by cells of the immune system in culture and is required to support optimal lymphocyte proliferation and production of cytokines by lymphocytes and macrophages. Glutamine may stimulate the immune system in mucosal tissue (Calder and Yaqoob, 1999). For example, in infected, early-weaned pigs, supplemental dietary glutamine (+40 g/kg diet) restored lymphocyte function and intramuscular glutamine concentrations (Yoo *et al*., 1997). Locally, supplemental dietary glutamine (+5 g/kg diet) increased ileal mucosa densities of macrophages and intra-epithelial lymphocytes (Domeneghini *et al*., 2004). Enteral glutamine supplementation (+45 g/kg diet) of rats with gut-derived sepsis enhanced peritoneal macrophage phagocytic activity (Yeh *et al*., 2004), preserved CD4+ T cells and maintained the pool of circulating T lymphocytes. In piglets, glutamine supplementation (+40 g/kg diet) of piglets stimulated cell proliferation to mitogens and Th-1-type cytokine response of T lymphocytes present in mesenteric lymph node cells (Johnson *et al*., 2006). Whilst it could be argued that such responses can be beneficial in the face of mucosal infection, it is not clear whether the immuno-stimulation by glutamine is direct, or indirect via the extra energy provided to the immune system (Bannink *et al*., 2006). Finally, glutamine supplementation reduced blood cortisol on the first day post weaning in pigs (Zhou *et al*., 2006).

Tryptophan is the most potent AA for stimulating cholecystokinin secretion and subsequent pancreatic enzyme production (Massey *et al*., 1998). Of the total body serotonin, 80% is located within the gut and dietary tryptophan may locally boost the serotoninergic system in the GIT and as such enhance peristaltic activity by smooth muscle and stimulate intestinal secretion by enterocytes (Spiller, 2001). The tryptophan-driven effect on gut serotonin may be (partly) responsible for the observed increases in digestion and/or absorption. In addition, supplemental dietary tryptophan (+5 g/kg diet) may increase gut functionality indirectly through its inhibitory effect on the peripheral sympathetic nervous system (Koopmans *et al*., 2005). Supplemental dietary tryptophan (+5 g/kg diet) has been shown to reduce villous atrophy in weaning piglets (Koopmans *et al*., 2006), to control the inflammatory response and to preserve growth in weaned pigs submitted to immune stress. Adequate dietary tryptophan (20% tryptophan to lysine ratio) reduced plasma haptoglobin concentrations, while its catabolism increased under indoleamine-2,3-dioxygenase activity during chronic lung inflammation in pigs (Le Floc'h *et al*., 2004; Melchior *et al*., 2004 and 2005). This highlights that during inflammatory stress in the gut, the tryptophan requirement to maintain growth could be greater than during 'health'. Indeed, supplementing a standard weaning diet with 100 mg/kg L-tryptophan improved daily gain

and also stimulated feed intake in piglets susceptible to intestinal adhesion of *E. coli* K88 during the first 4 days post challenge with this pathogen strain (Trevisi *et al.*, 2008). Tryptophan, via its conversion to serotonin, has been shown to reduce cortisol and noradrenaline concentrations in blood. In addition, surplus dietary tryptophan (+5 g/kg diet) enhanced the recovery from the 'social stress' of mixing piglets at weanling (Koopmans *et al.*, 2005 and 2006). The protective effect of tryptophan against stress may spare arginine and glutamine catabolism in enterocytes of post-weaning pigs since cortisol stimulates the degradation of arginine and glutamine (Flynn and Wu, 1997). Inhibition of the adreno-cortical and sympatho-adrenal axis activity by tryptophan and arginine during periods of stress (e.g. post weaning) may contribute to improved gut integrity, digestion and tissue anabolism.

Arginine has been used as a therapeutic agent for the treatment of necrotic enterocolitis, an inflammation of the gut induced by enterotoxins. Arginine-mediated production of nitric oxide (NO) causes smooth muscle relaxation, protects the gut from blood-borne toxins and tissue-destructive mediators (Di Lorenzo *et al.*, 1995; Di Lorenzo and Krantis, 2002), and enhances wound healing (Shi *et al.*, 2000). Supplementation of diets with ornithine, a metabolite of arginine, increased villous height and crypt depth in the jejunum and ileum of starved rats (Cynober, 1994). Supplemental dietary arginine (or glutamine) has also been reported to reduce bacterial translocation and to decrease atrophy of intestinal villi in rats with obstructive jaundice (Zulfikaroglu *et al.*, 2003). In weaned pigs challenged with lipopolysaccharide (LPS), arginine supplementation (at 5 and 10 g/kg feed) in weaned pigs partly protected from changes in villous–crypt architecture, in crypt cell proliferation and villous cell apoptosis induced by LPS (Liu *et al.*, 2008). Arginine supplementation to septic pigs restored the intestinal motility pattern and improved microcirculation and protein anabolism (Luiking *et al.*, 2005). Arginine (264 mg of N/kg rat per day) improves histone and acute-phase protein synthesis during Gram-negative sepsis (Léon *et al.*, 1991), stimulates the bactericidal actions of macrophages (Massey *et al.*, 1998) and restores depressed immunity (Cynober, 1994). Enteral arginine supplementation enhanced macrophage phagocytic activity and reduced total bacterial counts in the peritoneum of septic rats (Wang *et al.*, 2003; Yeh *et al.*, 2004).

Mucus is a semi-solid gel, which creates an important barrier for microorganisms and their toxins in the lumen of the gut. Cysteine and threonine are important building blocks for mucin; consequently, disease-induced stimulation in mucus secretion leads to extra requirement for cysteine and threonine (Lobley and Lapierre, 2003; Bannink *et al.*, 2006). Similarly, diets that stimulate mucin production decrease pig threonine retention (Myrie *et al.*, 2003), indicating that in this case the AA requirement should be adjusted. However, an excess of threonine has a negative effect on intestinal protein synthesis (Wang *et al.*, 2007), and this may be explained by its possible competitive inhibition on the absorption of other indispensable AA. Conversely, a threonine-deficient diet (6.5 *v.* 9.3 g/kg feed) increased intestinal para-cellular permeability and reduced villous height, villous height to crypt depth ratio and aminopeptidase N activity in the ileum of young piglets (Hamard *et al.*, 2007a and 2007b). Whilst these data highlight the functional role of threonine at the intestinal level, no studies have specifically assessed threonine requirements in piglets stressed with enterobacteria or reared in dirty environment. Therefore, in practice, the recommended values should be over the NRC (1998) requirement, but not increased up to 150% of this value (Wang *et al.*, 2007). Finally, cysteine provided at 25% above the NRC for pigs decreased jejunal mass, possibly due to its mucolytic properties (Harte *et al.*, 2003).

Spray-dried animal plasma

SDP is an abattoir by-product obtained from animal blood after exclusion of cells, concentration and spray drying. Three types of SDP products, from porcine (SDPP), bovine (SDBP) and unknown or mixed animal origin (SDAP) are available commercially. Following the temporary ban in Europe as a consequence of the bovine spongiform encephalopathy crisis, feeding SDPP is now allowed for pigs. Hygienic collection and processing of the blood are essential, and irradiation or formaldehyde treatment of SDP improved growth only in poor-quality SDP containing a high number of bacteria (DeRouchey *et al.*, 2004). The possibility that plasma may provide a source for the transmission of infectious diseases has also been considered (van Dijk *et al.*, 2001), and spray drying process has been shown to be effective in inactivating pseudorabies and porcine respiratory and reproductive syndrome viruses (Polo *et al.*, 2005).

It is now well established that both porcine and bovine SDP incorporated into weaning diets stimulate growth performance and feed intake (Coffey and Cromwell, 2001; van Dijk *et al.*, 2001), and that SDPP was more effective than SDBP (Pierce *et al.*, 2005). This observation could suggest some degree of specificity in the effect of IgG in SDPP against porcine pathogens. Pierce *et al.* (2005) showed that it was the immunoglobulin-rich fraction that was responsible for the beneficial effects of SDP. These effects are greater in piglets weaned at 22 days as compared to 32 days (Torrallardona *et al.*, 2002) and higher under poorer health conditions. This supports the view that the protective effect of SDP may be via the immune system or directly against pathogens (Coffey and Cromwell, 1995; Bergstrom *et al.*, 1997). Indeed, SDP lowered the incidence of diarrhoea in pigs challenged with pathogens (Owusu-Asiedu *et al.*, 2003a and 2003b; Conde, 2005) and improved body condition (Van Dijk *et al.*, 2002) and performance (Table 1).

It is widely accepted that the beneficial effects of plasma are mediated by its immunoglobulin fraction and their inhibitory activities against pathogens and enterotoxins. Therefore, differences in IgG contents (both quantitative and qualitative) may influence the efficacy of different SDP sources. SDP with guaranteed high levels of immunoglobulins were shown to be superior to conventional plasma

(Bosi *et al*., 2001; Conde, 2005). Furthermore, Bosi *et al*. (2001 and 2004) observed that challenge of piglets with *E. coli* K88 resulted in a lower concentration of specific IgA anti-K88 in plasma and saliva if they were fed a plasma source rich in IgG, suggesting a protective effect against the adhesion of *E. coli* K88 to the enterocytes.

A lower basal activation of the immune system was observed in piglets fed SDP than in piglets without SDP (Touchette *et al*., 2002). This is in line with the reduced production of pro-inflammatory cytokines (tumour necrosis factor-α, interleukin-8 and interferon-γ) in the jejunum of SDP-fed piglets challenged with enterotoxigenic *E. coli* (ETEC) K88 (Bosi *et al*., 2004). Porcine plasma was found to have levels of specific ETEC antibodies higher than SDAP of mixed origin (Owusu-Asiedu *et al*., 2002), and when tested in *E. coli* F18-challenged piglets, the source with more *E. coli* F18 antibodies (SDPP) showed numerically higher growth and feed intake. In contrast, other studies with piglets fed a plasma source without specific immunoglobulins for *E. coli* F18 have shown that SDP impedes *E. coli* F18 binding to the enterocytes by receptor competition by a non-specific protection mechanism (Nollet *et al*., 1999). Finally, hyper-immune SDPP obtained from pigs vaccinated against specific pathogens resulted in little production advantages over conventional SDPP in piglets challenged with the same pathogen (Conde, 2005; Niewold *et al*., 2007). This could suggest that non-specific mechanisms may play a role or that pre-existing antibodies may already be present in conventional SDPP as a consequence of 'natural' challenge.

Studies evaluating SDP in the presence or absence of antimicrobial medication have shown no (Coffey and Cromwell, 1995; Torrallardona *et al*., 2002; Bosi *et al*., 2004) or little (Torrallardona *et al*., 2003; Bikker *et al*., 2004) interactions between SDP and antimicrobials. These observations would suggest that SDP and antimicrobials might have essentially different modes of action and additive effects. This may be due to differences in antimicrobial efficacy against different pathogens. For example, SDP may not contain IgG against a particular pathogen that is sensitive to the antibiotic tested whereas SDP may be effective against pathogens that are resistant to the antibiotics. In addition, it must be considered that besides IgG, SDP contains growth factors, cytokines and other biologically active compounds that may also contribute to its positive effects on performance. Finally, SDP may reduce both the influx of LPS from the gut and LPS plasma levels, as shown with bovine colostrum in patients with Gram-negative bacterial infections (Struff and Sprotte, 2008).

In most studies in which SDP was compared directly to antibiotics, OA or other sources of immunoglobulins, SDP resulted in superior or equivalent performance (Coffey and Cromwell, 1995; Owusu-Asiedu *et al*., 2002; Torrallardona *et al*., 2002, 2003 and 2007b; Owusu-Asiedu *et al*., 2003a and 2003b; Bikker *et al*., 2004; Bosi *et al*., 2004; Conde, 2005; Pierce *et al*., 2005; Nofrarias *et al*., 2006).

Early studies argued that the effect of SDP is mediated by an increase in feed intake due to the improved palatability of the diets. This was supported by a double choice feed preference study in which piglets preferred a diet containing SDP to a diet containing dried skimmed milk (Ermer *et al*., 1994). However, the higher feed intake can also be related to the improved health and body weight of the piglets. Piglets pair-fed a feed with or without plasma protein have shown that its effects are independent of intake, suggesting a specific biological effect (Jiang *et al*., 2000b). Protein utilisation was improved by SDP in piglets and this could be explained by a lower protein catabolism by the microbiota (Jiang *et al*., 2000a and 2000b). SDP reduced intestinal mass and the cellularity of the lamina propia, suggestive of an antimicrobial activity, but data directly addressing the effects of SDPP on gut bacteria are scarce. In one study, it was shown to increase the number of lactobacilli in the ileal and caecal contents of piglets (Torrallardona *et al*., 2003), but this could not be confirmed in others (Conde, 2005; Torrallardona *et al*., 2007b). Finally, SDP may (Owusu-Asiedu *et al*., 2003a and 2003b) or may not (Jiang *et al*., 2000b; Owusu-Asiedu *et al*., 2002; Torrallardona *et al*., 2003; Nofrarias *et al*., 2006; Torrallardona *et al*., 2007b) increase villous height in the jejunum of pigs.

It can be concluded that SDP is a protein source of high interest for pre-starter piglet diets. Besides its clear positive effects on growth, feed intake and feed efficiency, there is enough evidence to support that SDP (mainly its IgG fraction) prevents the binding of pathogens to the gut wall and reduces the incidence of post-weaning diarrhoea. Therefore, SDP can be put forward as an alternative to in-feed antimicrobials for weanling piglets.

Bovine colostrum

Bovine colostrum is a by-product of milk production, and earlier studies on its inclusion in weaner diets led to increased growth performance and reduced days to slaughter (Pluske *et al*., 1999). Such an effect on growth was associated with a drastic enhancement of feed intake post-weaning when bovine colostrum was provided at 40 g/kg starter feed to pigs (Le Huërou-Luron *et al*., 2004; Boudry *et al*., 2008). Interestingly, the positive effect of colostrum lasted for 5 weeks after the end of the supplementation (Le Huërou-Luron *et al*., 2004). In another study, when the pigs were pair-fed the colostrum-supplemented and the control diets, the only significant effects of colostrum supplementation were a decreased gastric pH at 1 and 2 weeks post-weaning and an increased duodenal lactobacilli to coliform ratio caused by numerically lower coliform counts (Huguet *et al*., 2006). This would suggest that colostrum supplementation might predominantly act by stimulating appetite post weaning. Intestinal explants incubated in media with or without colostrum revealed that it may stimulate the expression of genes involved in epithelial cell migration along the crypt–villous axis and genes bearing anti-apoptotic properties (Huguet *et al*., 2007). In contrast with these results, Boudry *et al*. (2007a and 2007b), studying the effect of the dose of bovine colostrum

fed to pigs (0, 1 or 5 g/pig per day) for 21 days after weaning, did not observe any effect on feed intake and growth rate. The effects of bovine colostrum on immunity were unclear in these latter studies.

Plant extracts with anti-bacterial activities

Herbs have been in use in human nutrition for thousands of years due to renowned antiseptic qualities (Cowan, 1999). Numerous plants, their extracts or other natural substances possess anti-bacterial activity. Research has focused on natural components with antimicrobial activity as this was thought to be one of the modes of actions of AGP. Here we focus on essential oils from oregano, thyme, clove and cinnamon as increasing interest in feed science is given to these components.

Essential oils – occurrence and composition

Volatile or essential oils, distilled from non-woody parts of herbs, contain principally terpenoids and minor constituents including various aliphatic hydrocarbons, acids, alcohols, aldehydes and other compounds (Dorman, 1999; Bozin *et al*., 2006). The composition of the oils is highly variable within plants due to different environmental and climatic conditions (Table 3). The distillation method also influences the final composition of essential oils (Yang *et al*., 2007). Oregano oil (*Origanum vulgare*) and thyme oil (*Thymus vulgaris*) contain mainly carvacrol and thymol (Table 3) (Peñalver *et al*., 2005; Bozin *et al*., 2006). Eugenol dominates in oils from clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) (Dušan *et al*., 2006). Cinnamaldehyde is the main component of *C. verum* or *C. cassia* (Ooi *et al*., 2006). Essential oils themselves make 1.5% to 4.5% of the plant (Bozin *et al*., 2006; Hazzit *et al*., 2006; Yang *et al*., 2007).

In vitro *activity of essential oils*

The minimal inhibitory concentration (MIC) of carvacrol and essential oils from *O. dubium* against different bacterial strains (Table 4) was determined to be the same for pure component and the extract (Karioti *et al*., 2006). The MIC of essential oils from *O. vulgare* and *T. zygis* against several *Enterobacteriaceae* strains isolated from poultry and pigs with clinical disease outcome or carrier state were shown to vary widely, the highest MIC being observed for *E. coli* strains (Peñalver *et al*., 2005). Hammer *et al*. (1999) obtained MIC for *O. vulgare* essential oil against several Gram-positive and Gram-negative bacteria and *Candida albicans*. *Pseudomonas aeruginosa* was the least sensitive bacterium, having the highest MIC. The authors recorded similar values for essential oils from *S. aromaticum* and *T. vulgaris*. The MIC for clove and thyme essential oils against *S. aureus* and *E. coli* were half the minimal bactericidal concentration (Hammer *et al*., 1999).

Table 3 *Main components of selected essential oils (%)*

	Carvacrol	Thymol	Eugenol	Cinnamaldehyde	Reference
Oregano oil					
From *Origanum vulgare*	61	14			Bozin *et al*. (2006)
	76	nd			Peñalver *et al*. (2005)
	55	nd			Dušan *et al*. (2006)
	80	2.5			Sivropoulou *et al*. (1996)
From *O. floribundum*[a]	30	8			Hazzit *et al*. (2006)
From *O. floribundum*[b]	2	28			Hazzit *et al*. (2006)
From *O. glandulosum*[c]	1	24			Hazzit *et al*. (2006)
From *O. glandulosum*[d]	8	36			Hazzit *et al*. (2006)
From *O. Dubium*	70–71	0.1–0.3			Karioti *et al*. (2006)
Thyme oil					
From *Thymus vulgaris*	6	48			Bozin *et al*. (2006)
	nd	24			Dušan *et al*. (2006)
From *T. zygis*	2	50			Peñalver *et al*. (2005)
From *T. munbyanus*	8	38			Hazzit *et al*. (2006)
From *T. guyonii*	4	11			Hazzit *et al*. (2006)
From *T. pallescens*	42	0.1			Hazzit *et al*. (2006)
From *T. numidicus*	7	15			Hazzit *et al*. (2006)
Clove oil					
From *Syzygium aromaticum*			85		Dušan *et al*. (2006)
Cinnamon oil					
From *Cinnamomum zeylanicum*			77		Dušan *et al*. (2006)
From *C. verum* and *C. cassia*				85	Ooi *et al*. (2006)

nd: no data in reference.
[a]Collected in Chrea National Park.
[b]Collected in Hammam Melouane.
[c]Collected in Souhane.
[d]Collected in Ighzer mokrane.

Table 4 *Antimicrobial activity of selected essential oils* in vitro

	Carvacrol	Cinnamon oil	Cinnamaldehyde	Eugenol	Thymol	Oregano oil	Thyme oil	Clove oil	Reference
MBC[a]		$3–10 \times 10^3$						10 500	Cava *et al.* (2007)
	100–283	100–133		300–466	100–233				Si *et al.* (2006a and 2006b)
						156–625	156–2500		Burt and Reinders (2003)
	3.10–50					3.12–50			Karioti *et al.* (2006)
						1–200			Sivropoulou *et al.* (1996)
MIC[b]		500–3000						$3–4 \times 10^3$	Cava *et al.* (2007)
	3.12–50					3.12–50			Karioti *et al.* (2006)
	55.5–274.5	500		410			500	500	Dušan *et al.* (2006)
						2500–40 000	5000–40 000		Peñalver *et al.* (2005)
						1200–20 000	300–20 000	1200–25 000	Hammer *et al.* (1999)
		800–3200						1600–6400	Prabuseenivasan *et al.* (2006)
		75–600	75–600						Ooi *et al.* (2006)
MEC[c]	103–286		83–176	207–496	117–323				Michiels *et al.* (2007)

[a]MBC (μg/ml): minimal bactericidal concentration.
[b]MIC (μg/ml): minimal inhibitory concentration.
[c]MEC (μg/ml): minimal effect concentration.
The reader is asked to look in references for details.

Essential oils extracted from *O. vulgare* and *T. vulgaris* (Table 3) showed activity against Gram-positive and Gram-negative bacteria (Table 5) and their strains multi-resistant to antibiotics (Bozin *et al.*, 2006). Generally, Gram-positive bacteria seem to be more sensitive to essential oils than Gram-negative bacteria. Dorman and Deans (2000) determined equal anti-bacterial activity of volatile oils distilled from *O. vulgare* ssp. hirtum and *S. aromaticum* (clove) against several Gram-positive and Gram-negative bacteria, whereas essential oils from thyme showed stronger activity against Gram-positive organisms. The widest spectrum of activity was found for *T. vulgaris*, followed by oregano and clove essential oils. The pure components showed reduced activity against a wide spectrum of bacteria: thymol $>$ carvacrol $>$ eugenol. Hazzit *et al.* (2006) observed little inhibition of *Listeria monocytogenes* growth caused by essential oils from different oregano and thyme species. By contrast, oregano and thyme extracts were found to be inhibitory against several *Bacillus* sp. (Özcan *et al.*, 2006). Cinnamon and clove oils have shown highly variable MIC across bacteria (Prabuseenivasan *et al.*, 2006, Table 4). Clove oil was less active than cinnamon oil.

Oregano oil containing 74% carvacrol showed high antibacterial and antifungal activity and considerable reduction in bacterial growth even at very low concentrations (Sivropoulou *et al.*, 1996; Adam *et al.*, 1998). Essential oils from *C. verum* and *C. cassia* and pure cinnamaldehyde were inhibitory for bacteria (Table 5), yeasts (*C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei*), for moulds and dermatophytes (Ooi *et al.*, 2006). Also Matan *et al.* (2006) found that the antifungal activity of cinnamon and clove oils volatile against spoilage fungi, yeasts and bacteria. Park *et al.* (2007) found eugenol to be the active component of clove oil against several dermatophytes.

Extrapolation of in vitro *results to* in vivo

Simulation of gastric environment (artificial substrate containing several different sugars, casein, soybean oil, vitamins, minerals, etc., in a buffer solution of pH 3) to test anaerobic bacteria inhibition by essential oils revealed relatively high minimal effect concentration (MEC) for carvacrol, thymol, eugenol and *trans*-cinnamaldehyde (Michiels *et al.*, 2007). *Trans*-cinnamaldehyde displayed a strong inhibitory activity in the small intestinal environment (containing the same substrate as for the gastric environment with bile salts in a buffer of pH 6.5 inoculated with fresh small intestine supernatant of a weaned piglet). A synergistic action of carvacrol and thymol against total anaerobic bacteria was observed at ratios >1, strongly suggesting a different, dose-dependent mode of action of the essential oil components against *E. coli* and other coliform bacteria. Si *et al.* (2006b) noticed strong anti-bacterial activity of carvacrol, thymol, cinnamon oil and eugenol *in vitro* (Table 4). When added to pig caecal digesta, all the tested oils reduced the growth of the indigenous *E. coli*, exogenous *E. coli* O157:H7 when added and coliforms present in the digesta, with no effect on lactobacilli (Si *et al.*, 2006b).

Table 5 *Bacterial species inhibited in* in vitro *studies when plant essential oils or pure oil components were added to growth medium*

Bacterial species	Essential oil source or pure component	Reference
Acinetobacter baumanii	*Oregano vulgare*, *Syzygium aromaticum*, *Thymus vulgaris*	Hammer *et al*. (1999)
Acinetobacter calcoacetica	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Aeromonas hydrophila	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Aeromonas sobria	*O. vulgare*, *S. aromaticum*, *T. vulgaris*	Hammer *et al*. (1999)
Agrobacterium tumefaciens	Carvacrol	Karioti *et al*. (2006)
Alcaligenes faecalis	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Bacillus amyloliquefaciens	*O. vulgare*, *Thymbra sintensi* (thyme)	Özcan *et al*. (2006)
B. brevis	*O. vulgare*, *T. sintensi*	Özcan *et al*. (2006)
B. cereus	*O. vulgare*, *T. sintensi*, carvacrol	Karioti *et al*. (2006)
		Özcan *et al*. (2006)
B. megaterium	*O. vulgare*, *T. sintensi*	Özcan *et al*. (2006)
B. subtilis	*Cinnamonum zeylanicum*, *Eugenia caryophyllus* (clove), *O. vulgare*, *T. sintensi*, *T. vulgare*, *S. aromaticum*, carvacrol, eugenol, thymol	Sivropoulou *et al*. (1996)
		Dorman and Deans (2000)
		Bozin *et al*. (2006)
		Özcan *et al*. (2006)
		Prabuseenivasan *et al*. (2006)
B. subtilis var. *niger*	*O. vulgare*, *T. sintensi*	Özcan *et al*. (2006)
Beneckea natriegens	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Bifidobacterium longum	Thymol	Si *et al*. (2006a)
B. breve	Thymol	Si *et al*. (2006a)
Brevibacterium linens	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Brocothris thermosphacta	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Citrobacter freundii	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Clostridium sporogenes	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Enterococcus faecalis	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Hammer *et al*. (1999)
		Dorman and Deans (2000)
Enterobacter aerogenes	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Erwinia carotovora	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Escherichia coli (several strains, including K88, O157:H7, ETEC)	*C. zeylanicum*, *E. caryophyllus*, *O. vulgare*, *S. aromaticum*, *Thymus mastichina*, *T. vulgaris*, *Thymus zygis*, carvacrol, cinnamon oil, clove oil, eugenol, thymol	Sivropoulou *et al*. (1996)
		Hammer *et al*. (1999)
		Dorman and Deans (2000)
		Burt and Reinders (2003)
		Peñalver *et al*. (2005)
		Si *et al*. (2006b)
		Bozin *et al*. (2006)
		Dušan *et al*. (2006)
		Karioti *et al*. (2006)
		Prabuseenivasan *et al*. (2006)
Flavobacterium suaveolens	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Klebsiella pneumoniae	*C. zeylanicum*, *E. caryophyllus*, *O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Hammer *et al*. (1999)
		Dorman and Deans (2000)
		Prabuseenivasan *et al*. (2006)
Lactobacillus acidophilus	Carvacrol, cinnamon oil, thymol	Si *et al*. (2006a)
L. plantarum	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol, thymol	Dorman and Deans (2000)
		Si *et al*. (2006a)
Leuconostoc cremonis	*O. vulgare*, *S. aromaticum*, *T. vulgaris*	Dorman and Deans (2000)
Listeria monocytogenes	*O. floribundum*, *O. glandulosum*, *Thymus guyonii*, *T. munbyanus*, *T. numidicus*, *T. pallescens*	Hazzit *et al*. (2006)
Micrococcus flavus	*O. vulgare*, *T. vulgaris*	Bozin *et al*. (2006)
Micrococcus luteus	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
		Karioti *et al*. (2006)
Moraxella sp.	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
Proteus mirabilis	Carvacrol	Karioti *et al*. (2006)

Table 5 *Continued*

Bacterial species	Essential oil source or pure component	Reference
Proteus vulgaris	*C. zeylanicum*, *E. caryophyllus*, *O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
		Prabuseenivasan *et al.* (2006)
Pseudomonas aeruginosa	*C. zeylanicum*, *E. caryophyllus*, *O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol, thymol	Sivropoulou *et al.* (1996)
		Hammer *et al.* (1999)
		Dorman and Deans (2000)
		Bozin *et al.* (2006)
		Karioti *et al.* (2006)
		Prabuseenivasan *et al.* (2006)
P. talassi	Carvacrol	Karioti *et al.* (2006)
Rhizobium leguminosarum	*O. vulgare*, carvacrol, thymol	Sivropoulou *et al.* (1996)
Salmonella choleraesuis	*O. vulgare*, *T. Mastichina*, *T. zygis*	Peñalver *et al.* (2005)
S. enteritidis	*O. vulgare*, *T. mastichina*, *T. vulgaris*, *T. zygis*, carvacrol	Peñalver *et al.* (2005)
		Bozin *et al.* (2006)
		Karioti *et al.* (2006)
S. essen	*O. vulgare*, *T. mastichina*, *T. zygis*	Peñalver *et al.* (2005)
S. pullorum	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)
S. typhi	*O. vulgare*, *T. vulgaris*	Bozin *et al.* (2006)
S. typhimurium	*O. vulgare*, *S. aromaticum*, *T. mastichina*, *T. vulgaris*, *T. zygis*, carvacrol cinnamaldehyde, cinnamon oil, clove oil, eugenol, thymol	Sivropoulou *et al.* (1996)
		Hammer *et al.* (1999)
		Peñalver *et al.* (2005)
		Si *et al.* (2006a and 2006b)
Sarcina lutea	*O. vulgare*, *T. vulgaris,* carvacrol	Bozin *et al.* (2006)
		Karioti *et al.* (2006)
Serratia marcescens	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Hammer *et al.* (1999)
		Dorman and Deans (2000)
Shigella sonei	*O. vulgare*, *T. vulgaris*	Bozin *et al.* (2006)
Staphylococcus aureus	*C. zeylanicum*, *E. caryophyllus*, *O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol, thymol	Sivropoulou *et al.* (1996)
		Dorman and Deans (2000)
		Hammer *et al.* (1999)
		Bozin *et al.* (2006)
		Karioti *et al.* (2006)
		Prabuseenivasan *et al.* (2006)
S. epidermidis	*O. vulgare*, *T. vulgaris*	Bozin *et al.* (2006)
Yersinia enterocolitica	*O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol	Dorman and Deans (2000)

This study clearly showed no detrimental effect of pig digesta on the anti-bacterial essential oil activity *in vitro*.

It is difficult to relate the minimum effective dose *in vitro* to the dietary dose required in animals. In an *in vitro* study of the inhibition of *S. enterica* serovar Typhimurium, it was shown that the dose required for thymol was 700-fold greater than that required with the antibiotic gentamycin (Merialdi, personal communication), which was also able to inhibit *Salmonella* translocation to pig mesenteric lymph nodes (Modesto *et al.*, 2007). Based on the concentration of gentamycin required to protect weaning pigs, the amount of thymol required to be added to the diet has been estimated to be 4%.

The antimicrobial activity of essential oils *in vitro* is not always reflected in tests *in vivo*. For example, Si *et al.* (2006a and 2006b) showed that *Salmonella* shedding in challenged pigs was not reduced as the inhibitory effect of essential oils disappeared when they were mixed with the diet. This was probably because of binding of the phenolic and other components to fats and other hydrophobic materials of the diet. Emulsifiers like fenugreek or xanthan gum have no effect on essential oil activity and they are required for stabilising oils during storage (Si *et al.*, 2006a). However, whether emulsified components keep their activity when added to feed is not known. A possible way to overcome these problems could be micro-encapsulation of the oils before adding to the diet (Meunier *et al.*, 2007) and first studies considering this route have already been performed (Janczyk *et al.*, unpublished).

Feeding studies on weaning piglets

The published results of *in vivo* studies, whilst highly variable, suggest that relatively high concentrations of essential oils are required for displaying beneficial effects in young pigs (Table 6).

Table 6 *Average daily feed intake (ADFI), weight gain (average daily gain (ADG)) and feed conversion ratio (FCR) obtained in different studies on weaning piglets when herbs or herbal extracts were fed to the animals*

Supplement, its amount	ADFI (kg)	ADG (kg)	FCR	Weaned (days of age)	Mean BW at weaning (kg)	Duration of study (days)	Reference
Control	2.258[x]	0.187[x]	1.38	23	7.9	14	Kommera *et al.* (2006)
Antibiotic[a]	0.345[y]	0.255[y]	1.35	23	7.9	14	
PEP 1000-1[b], 1 g/kg	0.279[x]	0.199[x]	1.40	23	7.9	14	
Control	0.225	0.158	1.42	19	6.3	14	Kommera *et al.* (2006)
Antibiotic[a]	0.226	0.165	1.37	19	6.3	14	
PEP 1000-1[b], 4 g/kg	0.221	0.153	1.44	19	6.3	14	
Biotronic[c], 2 g/kg							
Control	0.263[x]	0.169[x]	1.56	21	5.4	14	Oetting *et al.* (2006)
Antibiotic[d]	0.316[y]	0.218[y]	1.45	21	5.4	14	
Herbal extract[e], 0.7 g/kg	0.261[x]	0.151[x]	1.73	21	5.4	14	
Herbal extract[e], 1.4 g/kg	0.239[x]	0.151[x]	1.87	21	5.4	14	
Herbal extract[e], 2.1 g/kg	0.282[x]	0.185[x]	1.52	21	5.4	14	
Control	0.597[x]	0.347[x]	1.72	21	5.4	35	Oetting *et al.* (2006)
Antibiotic[d]	0.674[y]	0.402[y]	1.71	21	5.4	35	
Herbal extract[e], 0.7 g/kg	0.590[x]	0.335[x]	1.76	21	5.4	35	
Herbal extract[e], 1.4 g/kg	0.538[x]	0.309[x]	1.74	21	5.4	35	
Herbal extract[e], 2.1 g/kg	0.607[x]	0.349[x]	1.74	21	5.4	35	
Control	0.413[x]	0.272[x] (last 7 days)	1.52	21	6.7	35	Sads and Bilkei (2003)
Oregpig[f], 1 g/kg	0.491[y]	0.372[y] (last 7 days)	1.32	21	5.9	35	
Oregpig, 1 g/kg	0.470[y]	0.359[y] (last 7 days)	1.31	21	5.6	35	
Control	0.693	0.452	1.53	20–21			Manzanilla *et al.* (2004)
Herbal extract[g], 0.15 g/kg	0.645	0.403	1.60	20–21	6.0 (8.1 at start)	21 (start 12 days pw)	
Herbal extract[g], 0.3 g/kg	0.645	0.423	1.52	20–21			
Control	0.453[x]	0.237[x]	1.91[x]	28	8.0	21	Molnar and Bilkei (2005)
Oregpig, 1 g/kg	0.453[x]	0.242[x]	1.87[y]	28	8.1	21	
Oregpig, 2 g/kg	0.482[y]	0.258[y]	1.87[y]	28	8.2	21	
Oregpig, 3 g/kg	0.475[y]	0.258[y]	1.84[z]	28	8.1	21	
Control	0.063 – week 1	0.040	1.58	16–19	4.9	28	Namkung *et al.* (2004)
	0.181[x] – week 2	0.121	1.50				
	0.469[x] – week 3	0.338[x]	1.39				
	0.765 – week 4	0.556	1.38				
Antibiotic[h]	0.068 – week 1	0.036	1.89	16–19	4.9	28	
	0.204[y] – week 2	0.165[x]	1.24				
	0.460[x] – week 3	0.334[x]	1.38				
	0.716 – week 4	0.509	1.41				

Table 6 *Continued*

Supplement, its amount	ADFI (kg)	ADG (kg)	FCR	Weaned (days of age)	Mean BW at weaning (kg)	Duration of study (days)	Reference
Herbal extract[i], 7.5 g/kg	0.056 – week 1	0.024	2.33	16–19	4.9	28	
	0.172[x] – week 2	0.114	1.51				
	0.388[y] – week 3	0.288[y]	1.35				
	0.637 – week 4	0.503	1.27				

[a]Carbadox 50 mg/kg diet.
[b]PEP-1000-1 – essential oil mix containing anis oil, citrus oil, oregano oil and natural flavours.
[c]Biotronic – organic and inorganic acids mix containing phosphoric and lactic acids.
[d]Zn-bacitracin, olaquindox, colistin, 50 mg/kg diet each.
[e]Herbal extract in this study contained 20% of essential oils: 3.3% clove oil, 3.3% eugenol, 3.3% oregano oil, 3.3% carvacrol and 6.7% thyme oil.
[f]Oregpig – 500 g oregano oil, 33.4 g thymol, dried oregano flowers and leaves ad 1000 g. Concentration of carvacrol – 60 g/kg, thymol – 55 g/kg.
[g]Herbal extract used in this study: carvacrol 50 g/kg, cinnamaldehyde 30 g/kg and capsicum oleoresin 20 g/kg.
[h]Lincomycin 110 mg/kg diet.
[i]Herbal extract used in this study contained extracts of cinnamon, thyme and oregano on clay carrier.
[x,y,z]Different superscripts mark significant difference between values in a column within one study.

Kommera *et al.* (2006) observed no effect on feed intake, growth performance or feed efficiency when a mix of essential oils was added to the diet (1 to 4 g/kg) of weaning pigs, without or with a combination of OA. Supplementation of piglet diet with 2.1 g/kg of herbal extract resulted in an improved growth performance similar to an antibiotic control group, with intermediate feed intake and feed efficiency (Oetting *et al.*, 2006). The only variables influenced by the highest dose tested were the apparent digestibility of DM and the weight of the small intestine, which were higher with herbal extract supplementation. Improved growth performance and feed efficiency were recorded in weaned piglets fed a product containing oregano extract at a dose of 1 g/kg (Sads and Bilkei, 2003). By contrast, no effect could be observed when 0.15 or 0.3 g/kg of herbal mix was fed to early-weaned piglets (Manzanilla *et al.*, 2004), except for a decreased gastric emptying rate (Manzanilla *et al.*, 2004) and a reduction in the lactobacilli to enterobacteria ratio in caecum at a dose of 0.3 g/kg (Castillo *et al.*, 2006).

Molnar and Bilkei (2005) conducted a study in a pig herd in Hungary where post-weaning colibacillosis had been present for years. Interestingly, the mortality rate decreased from 3.7% in the control to 2% in the group supplemented with 1 g/kg of a product containing oregano extract and to 1% in the groups supplemented with 2 and 3 g/kg ($P < 0.05$). An increase in growth performance was observed with high doses (2 or 3 g/kg, $P < 0.05$). A reduction in feed conversion was observed already at the level of 1 g/kg of this product ($P < 0.05$). Contrasting results were obtained by Namkung *et al.* (2004) who recorded a reduction in growth performance and feed intake in the piglets supplemented with 7.5 g/kg of herbal extract ($P < 0.05$) without effects on gut anatomy or immunity. However, coliform counts were reduced in the ileum and faeces ($P < 0.05$), but not in the colon.

Based on the published literature it is clear that the effective concentration of essential oils in feed still needs to be established. A recent study performed was therefore aimed towards this issue. The effect of thymol addition to a starter diet (10 g/kg), determined from *in vitro* test and toxicology information, was tested in pigs with or without *S. typhimurium* challenge (Trevisi *et al.*, 2007). The final body weight after 29 days of trial was not affected by thymol but feed intake was reduced. Thymol protected against a rise in body temperature 1-day post challenge, but it did not reduce faecal shedding of *Salmonella*. Thymol increased the density of acid-secreting cells in gastric oxintic glands. Bacterial diversity in the jejunum was also affected. *Actinobacillus minor* was present in almost all the pigs fed thymol, *Citrobacter freundii* being absent (Janczyk *et al.*, 2008).

In terms of palatability, Jugl-Chizzola *et al.* (2006) observed that piglets had a preference for diets with low inclusion rates of herbs in diets but they could not show differences between thyme and oregano.

Finally, the bioavailability and pharmacokinetics of essential oils have received little attention in pigs. Studies in

humans suggest a rapid absorption of these molecules (e.g. thymol, Kohlert *et al*., 2002). This could reduce the action of essential oils on commensal and exogenous microflora *in vivo*. On the other hand, different essential oils may be sensitive to oxidation and thus they may undergo degradation (Pérez-Alonso *et al*., 2008). Protection of essential oils, e.g. by micro-encapsulation, may be a valuable strategy in animal feeding (Meunier *et al*., 2006 and 2007), and more studies are clearly required in this area.

Conclusions and perspectives

Relating to the recent ban on in-feed antibiotics, many efforts have been made in the past years for optimising further diet composition in terms of protein, AA and energy. Additional substances including OA and compounds of plant origin with known antimicrobial properties have also been evaluated. It has become clear that diet supplementation with plasma protein is probably the best way for preventing post-weaning gut disorders, provided it is of high hygienic quality. Such a success lies mostly in its richness in immunoglobulins that act at two complementary levels: protecting the intestinal mucosa from luminal aggression and stimulating the immune system for defence against pathogens. Interestingly, the mode of action of plasma protein appears to be different from, and additive to, that of in-feed antibiotics. Positive results have been obtained with particular AA (Gln, Trp, Arg, Cys, Thr) probably because their requirements are increased during periods of general and immune stress, especially in younger animals and under poor hygienic conditions. Many OA have also proven successful, acting on gut ecology and preventing the outgrowth of pathogenic bacteria. Variable results have been obtained with SB administered orally. However, when it displays positive effects *in vivo*, its mechanisms of action are still unclear, thus calling for additional work. Investigations *in vitro* clearly show antimicrobial properties of essential oils. They are supported by one or two major molecules or by more subtle synergistic interactions within the extract mixture. However, major discrepancies do exist between *in vitro* and *in vivo* results, the latter being variable and rather inconsistent. It appears that the diet itself may neutralise the antimicrobial activity of essential oils *in vivo*. Finally, the bioavailability and pharmacokinetics of these essential oils in pigs are mostly unknown. This will undoubtedly constitute an area for future investigation.

In terms of cost effectiveness, this review in some way confirms the practices that are now more sprayed among the experts of piglet feed formulation: to include consistently more than one OA and to raise some AA when the gut environment is more challenged.

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