Phytogenics Improve First-line Defence in Swine and Poultry

PEER REVIEWED

Abstract
The gastro-intestinal tract (GIT) is the first line of defence in monogastric animals. Antimicrobial growth promoters (i.e. low dose levels of in-feed antibiotics) can limit the growth of potential pathogenic bacteria in the GIT and thereby reduce the incidence and severity of intestinal infections. Moreover, they control local inflammatory responses caused by these intestinal infections and consequently improve intestinal integrity and prevent loss of production performance. Phytogenic components, like monoterpenes from thyme, can also improve the GIT as first line of defence, as they reduce fatty acid oxidation in the enterocytes, reduce cell apoptosis, and therefore improve intestinal mucosa integrity. In addition, phytogenic substances stimulate secretion of immunoglobulins, limit local inflammations and stimulate phagocytic activity.

Introduction
Modern swine and poultry breeds have a high genetic performance potential that can only be realised in case of optimal housing conditions, high health status, low stress levels and good quality feeds. During the first weeks of life, the immune system of the animal is still immature. In combination with inadequate uptake of colostrum in piglets, or of maternal antibodies from the egg in birds, this will often result in increased morbidity and mortality. Stress factors like weaning, long-term fasting, abrupt dietary changes, long-distance transports, mixing of animals from different origins, high stocking densities, improper climate conditions, or suboptimal hygiene generally increase the susceptibility for infectious diseases in young animals. As the epithelia of the gastrointestinal and respiratory tract are the first contact sites for pathogens, optimising epithelial defence mechanisms is important to reduce incidence of pathogenic diseases. Table 1 shows four of the most important infectious diseases in piglets, leading to decreased production performance and welfare and increased economic losses (Ayrle *et al*., 2016).

Gastrointestinal disease complex	Pathogens group			Pathophysiology/ pathogenesis	Demands for prophylaxis and therapy
	Bacteria	**Viruses**	**Parasites**		
Neonatal Diarrhoea	*Escherichia coli*	Rotavirus	*Cryptosporidum* spp.	Secretory/ malabsortive/ maldigestive diarrhea	Antimicrobial
Post-weaning Diarrhoea	*Clostridium perfringens*	Coronavirus	Isospora suis	Enteritis	Antiviral
	Lawsonia intracellularis	Porcine Circovirus type 2	*Trichuris suis*	Colitis	Antidiarrhoeal
	Brachyspira spp.		*Oesophago-stomum dentatum*	Dehydration	Antiadhesive
	Salmonella spp.			Acidosis	Astringent
	(*Yersinia* spp.)			Septicaemia	Analgesic
				Neurological symptoms	Anti-inflammatory
				Apathy	Orexigenic
				Reduced growth rates	Prebiotic
					Immune stimulant

Table 1. Challenging infectious gastrointestinal diseases in piglets: Pathogens and pathophysiology/ pathogenesis resulting in demands for prophylaxis and therapy (Ayrle *et al*., 2016).

Infections with enterotoxigenic *E. coli* strains have been reported as main causes for a constant high morbidity and mortality in piglets, both pre- and post-weaning (Thomson and Friendship, 2012). The prevalence of post-weaning diarrhoea was reported to be 35 % in France (Madec *et al*., 1998), morbidity exceeded 50 % in Finland (Laine *et al*., 2008) whereas mortality can be as high as 25 % without therapy (Fairbrother *et al*., 2012), as reviewed by Ayrle *et al*. (2016). The incidence of neonatal diarrhoea mainly depends on the concentration of antibodies in sow's colostrum, whereas the risk for post-weaning diarrhoea increases after sudden dietary changes, reduced stomach acidity (high dietary buffering capacity) and inadequate secretion of gastric and pancreatic enzymes. Inefficient nutrient digestion and absorption in the proximal parts of the small intestine of young animals is known to induce dysbiosis (Teirlynck *et al*., 2011; Rossi *et al*., 2012). Hafez (2011) reviewed enteric diseases of poultry, with special focus on *Clostridium perfringens*. He concluded that the incidence of intestinal disorders was drastically increased after the ban on antimicrobial growth promoters. It is clear that a good understanding of these intestinal disorders is needed to be able to develop effective nutritional intervention strategies.

One of the intervention strategies can be based on phytogenic feed additives, as many different modes of action have been described in literature. These are, for example, the stimulation of the secretion of pancreatic and brush-border enzymes and the secretion of bile acids, as well as anti-oxidant, anti-inflammatory and/or anti-bacterial effects, or immunomodulating effects. Some of these effects are essential to the GIT as first line of defence and therefore can be used in strategies to replace antimicrobial growth promoters as in-feed antibiotics.

Essential Oils as Powerful Anti-oxidants Showing Anti-inflammatory Effects in the GIT
Essential oils can be powerful anti-oxidants by direct scavenging of intracellular oxygen radicals or by stimulating the production of anti-oxidant enzymes. Fatty acid oxidation in the enterocytes increases cell apoptosis, stimulates cell turnover, reduces intestinal integrity (Sun *et al*., 1998) and potentially increases bacterial translocation. The latter will result in macrophage recruitment and local inflammatory responses. Therefore, anti-oxidants potentially improve intestinal integrity. Thyme essential oils were shown to reduce fatty acid oxidation in the duodenal enterocytes in broilers, resulting in a direct improvement of the trans-epithelial electrical resistance, which indicated an improved intestinal barrier function (Placha *et al*., 2014). Mueller *et al*. (2012) showed the positive effect of thyme oil and other terpenes on anti-oxidant status in jejunal, colon and liver tissue samples. They tested the effect of broccoli sprouts extract (BE), turmeric oil (Cuo), oregano oil (Oo), thyme oil (To) and rosemary oil

(Ro) added to diets of four-week-old male weaned piglets for a four-week period (Figure 1). The anti-oxidant capacity in jejunal mucosa was significantly improved compared to the control by all terpenes, whereas liver values were numerically (for broccoli extract, turmeric oil, oregano oil and thyme oil) or significantly (for rosemary oil) increased.

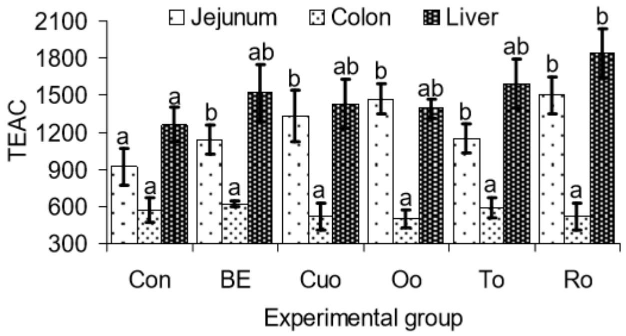

Figure 1. TROLOX equivalent antioxidant capacity (TEAC in µmol/g organ fresh matter and kg body weight) in jejunal mucosa, colon and the liver of piglets. Values represent means + SEM (n=8 per experimental group). Mean values with unlike superscripts indicate significant differences between means (P< 0,05) in the LSD test.

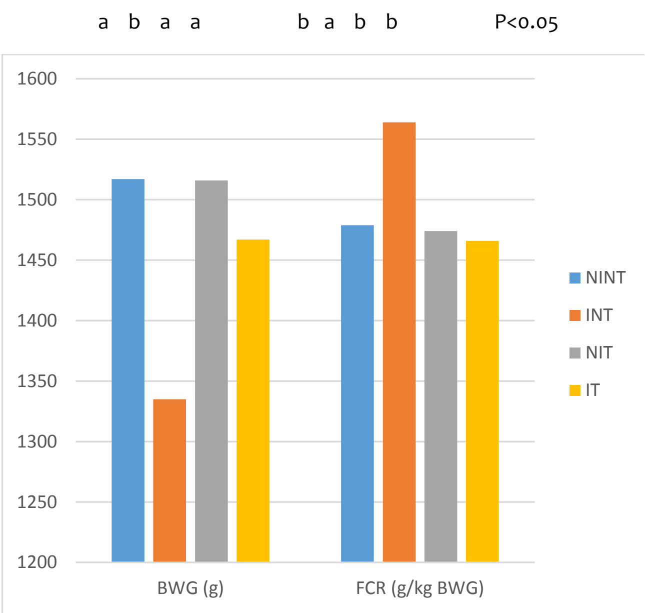

Figure 2. The effect of an anti-inflammatory agent during a Clostridium infection in broilers on production performance from 0-28 days of age (Van der Klis, 2012).

Legend for figure 2: NINT: Non-Infected and Non-Treated; INT: Infected (Day 9 oral inoculation with *Eimeria maxima* followed by oral inoculation with Cl. perfringens at Days 14, 15 and 16) and Non-Treated; NIT: Non-Infected and Treated (35 mg Na-salicylate/kg BW via drinking water); IT Infected and Treated.

Niewold (2007) hypothesised that part of the effects of antimicrobial growth promoters in monogastrics were of a non-antibiotic, anti-inflammatory nature. Van der Klis (2012) demonstrated that limiting (local) inflammatory responses in broilers by supplying aspirin via drinking water during sub-clinical necrotic enteritis, indeed almost eliminated the negative impact of the infection on production performance (Figure 2). Therefore, phytogenics with anti-inflammatory effects, like monoterpenes (De Cassia da Silveira *et al.*, 2013), most likely will have clear positive effects on production performance of broilers and pigs after intestinal health challenges, showing additional value compared to products that only have antibacterial effects. Some effects of essential oils on mRNA expression of various pro- and anti-inflammatory parameters in the colon of rats are given in Table 2 (Mueller *et al.*, 2013).

Gene	Period	Experimental group compared with untreated control rats				
		DSS	BE	Cuo	To	Ro
NFκB	Phase 1	—	↔	↔	↔	↔
	Phase 2	↔	↔	↔	↔	↔
	Phase 3	↑	↔	↔	↔	↔
TNFα	Phase 1	—	↔	↔	↔	↔
	Phase 2	↔	↔	↔	↑	↔
	Phase 3	↑	↑	↑	↑	↑
COX2	Phase 1	—	↔	↔	↔	↔
	Phase 2	↑↑↑	↔	↔	↑	↔
	Phase 3	↔	↔	↔	↔	↔
IL-1β	Phase 1	—	↔	↔	↔	↔
	Phase 2	↔	↔	↔	↔	↔
	Phase 3	↑↑	↔	↔	↔	↔
IL-10	Phase 1	—	↔	↔	↔	↔
	Phase 2	↔	↔	↑	↑↑↑	↑↑
	Phase 3	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2. Effect of various essential oils on mRNA expression of various pro- and anti-inflammatory parameters in the colon of dextran sulphate sodium- (DSS-) induced colitis in rats, compared to an untreated control (Mueller *et al.*, 2013). Different arrows summarise the effects on gene expression: ↔ no effect, ↔: increase, ↔: decrease; n.d.: not detectable. Phases: (1) pre-treatment phase (7 days), (2) DSS treatment phase (6 days), and (3) recovery phase (6 days).

The anti-inflammatory activity of limonene (LIM), the main constituent of the essential oil in *Citrus latifolia* Tanaka (CLEO), was investigated by Kummer *et al.* (2013). Limonene, one of the most common terpenes in nature, has been shown to have anti-inflammatory effects by reducing eosinophil chemotaxis. It effectively inhibits lipopolysaccharide-induced nitric oxide and prostaglandin E2 production in macrophages and decreased interleukin-1α(IL-1α) levels in normal human undifferentiated NCTC 2544 keratinocytes. In zymosan-induced peritonitis, LIM (500mg/kg) decreased the infiltration of peritoneal exudate leukocytes and decreased the number of poly-morphonuclear leukocytes. *In vitro* chemotaxis revealed that CLEO and LIM (1, 3, and 10µg/mL) promoted a significant reduction of neutrophil migration toward chemoattractant N-formyl methionyl leucyl phenylalanine (fMLP) and leukotriene B_4 (LTB_4). Limonene (500mg/kg) also reduced TNF-α levels but did not alter IL-10 levels in the peritoneal exudate. In conclusion, Kummer *et al.* (2013) showed that LIM isolated from CLEO had potential anti-inflammatory effects, most likely by inhibiting pro-inflammatory mediators present in inflammatory exudate and leukocyte chemotaxis via inflammatory cytokines-like TNF-α.

Immunomodulating Effects

Phytogenics also have immunomodulating effects. Dietary thymol supplementation enhanced serum IgA and IgM in piglets challenged with *S. typhimurium* after weaning and exhibited anti-inflammatory properties in the stomach. The latter effect was shown by reduced TNF-α mRNA levels in the stomach (Trevisi *et al.*, 2007). These piglet data were supported by Placha *et al.* (2014) in broilers who showed increased duodenal IgA levels and stimulated phagocytic activity in blood of broilers fed with *Thymus vulgaris* essential oil-supplemented diets. Apart from essential oils, triterpenoid saponins also stimulate humoral immune response in the intestinal tract as shown by Zhai *et al.* (2011, 2014). They showed positive effects of ginseng stem leaf saponins on IgA-producing cells in duodenal, jejunal and ileal tissue up to three weeks post-immunisation of leghorn chickens immunised with infectious bursal disease virus, followed by an increased number of intra-epithelial lymphocytes (Zhai *et al.*, 2014) indicating improved intestinal epithelial defence.

Several phytogenics components were effective in the transfer of immunity from lactating sows during lactation to suckling piglets. Hossain *et al.* (2015) showed a dose-dependent increased IgG content in blood serum of suckling piglets from sows fed fenugreek seed extract (FSE), resulting in a reduced *E. coli* excretion at weaning (Table 3). IgG is considered a key role in the prevention of transmission of pathogens in blood or body tissues.

IgG (mg dL^{-1}) in blood serum	CON	FSE1 (0.1%)	FSE2 (0.2%)	SEM	P-linear
sows farrowing	501	513	544	12.0	0.022
sows at weaning (d 21)	528	584	596	16.2	0.024
piglets suckling	292	298	349	16.4	0.013
E. coli excretion in sow faeces at weaning (log 10 CFU.g^{-1})	5.9	5.5	5.3	0.15	0.026

Table 3: The effect of increased levels of Fenugreek seeds extract (FSE) in the content of IgG (mg dL-1) in blood serum of sows at farrowing and at weaning and in suckling piglets (from Hossain *et al.*, 2015)

Similar effects were observed when weaned piglets were fed on 0.2 % FSE for 42 days (Begum *et al.*, 2016).

Conclusions

1. Essential oils can be powerful anti-oxidants by direct scavenging of intracellular oxygen radicals or by stimulating the production of anti-oxidant enzymes. Effects were demonstrated in vitro and in vivo.
2. Phytogenics with anti-inflammatory effects, like monoterpenes will have clear positive effects on production performance of broilers and pigs after intestinal health challenges.

3. Phytogenics have immune-stimulating effects improving vaccination response, number of IgA producing cells and intraepithelial lymphocytes.
4. Different modes of action shown stimulate the intestinal mucosa as first line of defence.
5. Carefully designed phytogenic feed additives, which are generally of a complex nature combining various actives, can let 'nature perform' to its full potential.

Cited literature for Delacon's Article "Phytogenics Improve First-line Defence in Swine and Poultry" by Ester Vinyeta and Jan Dirk von der Klis

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