Phytogenics Improve First-line Defence in Swine and Poultry

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, phytophagic 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).

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 phytophagic 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.

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 lipopolysacharide-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 B4 (LTB4). Limonene (500mg/kg) also reduced TNF-α levels but did not alter IL-10

**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).
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.

<table>
<thead>
<tr>
<th>IgG (mg dL⁻¹) in blood serum</th>
<th>CON</th>
<th>FSE (0.1%)</th>
<th>FSE (0.3%)</th>
<th>SEM</th>
<th>P-linear</th>
</tr>
</thead>
<tbody>
<tr>
<td>sows farrowing</td>
<td>501</td>
<td>513</td>
<td>544</td>
<td>12.0</td>
<td>0.002</td>
</tr>
<tr>
<td>sows at weaning (d 21)</td>
<td>538</td>
<td>584</td>
<td>596</td>
<td>16.2</td>
<td>0.084</td>
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<tr>
<td>piglets suckling</td>
<td>292</td>
<td>298</td>
<td>349</td>
<td>16.4</td>
<td>0.013</td>
</tr>
<tr>
<td><em>E. coli</em> excretion in sow faeces at weaning (log 10 CFU g⁻¹)</td>
<td>5.9</td>
<td>5.5</td>
<td>5.3</td>
<td>0.15</td>
<td>0.016</td>
</tr>
</tbody>
</table>

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 phytoalgenic 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

**References**


Jan Dirk van der Klis, Head of Products & Innovation, Species Leader Poultry
Jan Dirk van der Klis started his career in poultry nutrition in 1987 at Spelderholt Institute in the Netherlands and completed his Ph.D. in animal nutrition at the Wageningen Agricultural University in 1993. He worked for several research institutes in the Netherlands. Jan Dirk van der Klis developed the digestible phosphorus system for poultry in the Netherlands, which is currently adopted worldwide. He was involved in the early research on microbial phytase, and wrote the first scientific publication on the efficacy of phytase in laying hens. During his prior 8-year profession he was consulting nutritionists at feed mills worldwide. In 2014 he joined Delacon Biotechnik GmbH as Species Leader Poultry, in 2015 he has become Head of Products & Innovation.

Ester Vinyeta, Species Leader Swine
Ester Vinyeta started her professional career as lecturer and researcher on crop and food production at the Universitat de Vic (Barcelona, Spain) in 1992. In 2002 she moved to Esporç S.A., a Spanish feed company and swine integrator, as nutritionist and technical director of three feed mills. She conducted research partnering with universities and research institutes on feed technology, animal production and meat quality. In 2009 she joined Schothorst Feed Research as senior researcher and consultant, focusing on nutritional requirements of pigs and fattening pigs but also collaborating closely with the poultry team. In 2011, she joined Bühler AG as Animal Nutrition Manager and also gave trainings offered by the Swiss Feed Technology Institute (SFT). In 2014 she took over the part of Species Leader Swine and feed technology expert at Delacon.