Gut flora and immune modulation by plant extracts

by Chris Kamel and Abel Santos, Carotenoid Technologies, Pol. Ind. Francoli, 30, Tarragona 43006, Spain.

The use of plants and their extracts in animal feeds is not new with the first records of plant based traditional medicine systems dating back to approximately 2600BC from Mesopotamia.

In recent years there has been a surge of interest in the use of plants and their extracts as alternative performance enhancers for animals, largely due to the European Union Directive 1830/2003 banning the use of medicinal feed additives (in-feed antibiotics, and ionophores used as growth promotants) and consumer pressures for the use of 'natural' products in animal feeds.

The Asian pig market finds itself in a unique position to profit from the European situation which has helped focus on sustainable plant extracts for livestock feeding strategies.

It is estimated that there are 250,00-500,000 species of plants on earth. The number of marine based plants may be even greater. A relatively small number of these plants have been chemically characterised and identified as having functional characteristics. Only a handful have been demonstrated to be beneficial for enhancing production by ruminants. The focus of this article will be the use of plant based compounds as feed additives in diets fed to mature ruminants.

Form	Utilised parts	Main compound	Reported properties
Aromatic spices			
Nutmeg	Seed	Sabinene	Digestion stimulant, anti-diarrhoeic
Cinnamon	Bark	Cinnamaldehyde	Appetite/digestion stimulant, antiseptic
Clove	Cloves	Eugenol	Appetite/digestion stimulant, antiseptic
Cardamom	Seed	Cineol	Appetite/digestion stimulant
Coriander	Leaf, seed	Linalol	Digestion stimulant
Cumin	Seed	Cuminaldehyde	Digestive, carminatif, galactagogue
Anise	Fruit	Anethol	Digestion stimulant, galactagogue
Celery	Fruit, leaves	Phtalides	Appetite and digestion stimulant
Parsley	Leaf	Apiol	Appetite/digestion stimulant, antiseptic
Fenugreek	Seed	Trigonelline	Appetite stimulant
Pungent spices			
Capsicum	Fruit	Capsaicin	Anti-diarrhoeic, anti-inflammatory, stimulant, tonic
Pepper	Fruit	Piperine	Digestion stimulant
Horseradish	Root	Allyl isothiocyanate	Appetite stimulant
Mustard	Seed	Allyl isothiocyanate	Digestion stimulant
Ginger	Rhizome	Zingerone	Gastric stimulant
Aromatic herbs and spices			
Garlic	Bulb	Diallyl disulfide	Digestion stimulant, antiseptic
Rosemary	Leaf	Cineol	Digestion stimulant, antiseptic, antioxidant
Thyme	Whole plant	Thymol	Digestion stimulant, antiseptic, antioxidant
Sage	Leaf	Cineol	Digestion stimulant, antiseptic, carminatif
Bay laurel	Leaf	Cineol	Appetite/digestion stimulant, antiseptic
Peppermint	Leaf	Menthol	Appetite/digestion stimulant, antiseptic

Table 1. Common plant extracts and reported properties (Kamel, 2000).

The chemistry of plants

The use of plant compounds in livestock production has been termed ethnoveterinary medicine.

Ethnoveterinary medicine is the use of local or indigenous knowledge and methods for caring for, healing, and managing livestock. In addition to using plants for medicinal purposes, ethnoveterinary medicine includes social practices and ways in which livestock are managed in farming systems.

Plants have an almost infinite ability to synthesise compounds. Most of the functional compounds of interest are low molecular weight, secondary metabolites.

These compounds afford plants a competitive advantage by acting as defence mechanisms against predators and pathogens or environmental stress. Some are toxic to animals, but others may be beneficial.

Beneficial compounds of plant origin may be found in the following general categories:

- Herb – a flowering plant whose stem does not become woody.
- Spice – a pungent or aromatic substance of vegetable origin.
- Botanical – a drug made from part of a plant, such as the root, bark and leaves.
- Essential oil – variable mixtures of plant volatile compounds possessing a characteristic odour or property.

Bioactive compounds that have been chemically identified in a selected list of plants (see Table 1).

The complexity of identifying chemicals and their potential interactions have created considerable challenges for accurately defining mode of action for medicinal plants and combinations of plants. For example, oregano (Origanum vulgare) may have 30 or more chemicals that are anti-microbial.

Advances in phytochemistry and results of in-vitro and in-vivo studies demonstrate structure-function relationships for plant compounds. The transition from folklore to science has precipitated the commercial development of herb and botanical products for use as anti-microbial, anthelmintic, anti-oxidant, anti-inflammatory and immunostimulatory agents in young animal diets.

Unfortunately, there are few refereed studies supporting mode of action or efficacy of these products and the specific herbs and botanicals used in their preparation. Herbs and botanicals are generally classified by AAFCO (2001), as flavouring agents (spices, seasonings and essential oils, for example, origanum, anise, and ginger). Except in the case of the European Union, where European Directive 1830/2003 has been adopted for the future registration of plant extracts and their phytogenic active ingredients as feed additives, there are few restrictions on their use for the classified purpose.

Because of their classification as flavours, considerable interest exists for using plant compounds to improve or change the pattern of feed intake. A multitude of other effects has been observed in monogastric species fed plant compounds either singly or in combination.

Nutrient digestion and absorption may be affected by the effects of plant chemicals directly on gut tissues and digestive enzyme secretions or indirectly by stimulatory or inhibitory effects on microbial populations. Immune modulation, antioxidant and anti-inflammatory properties have been observed.

Limitless potential

An excellent review of physiologically active components of plant origin was presented by Rhodes (1996).

While their potential seems almost limitless, the sustainability of this class of feed additives has been called into question, however, due to the lack of salient information regarding their stability, efficacy and safety. This article will attempt to review the most promising phytoingredients and their application alone or together to

Continued on page 11

Continued from page 9
reduce stress challenge in young animals in today's livestock rearing practices. Trials in Europe are demonstrating the value of research based feed strategies for the protection of gut integrity in young pigs.

This information is timely, because the market has turned against antibacterial agents that have been used for decades to achieve a better control of pathogens and improved growth and viability.

Intestinal antioxidation

From the perspective of function, the contents of the gut lumen lie outside the body and contain a toxic/antigenic load from which the body needs to be protected.

Protection is supplied by complex mechanisms which support one another: intestinal secretions (primarily mucus and secretory IgA), the mucosal epithelium, and intramural lymphocytes. This primary, intestinal barrier is supported by the liver, through which all enterically derived substances must pass before entering the arterial circulation for transport to other tissues and organs.

Kupffer cells in the hepatic sinusoids remove absorbed macromolecules by phagocytosis. Hepatic microsomal enzymes alter gut-derived chemical substrates by oxidation and by conjugation to glycine and glutathione (GSH) for excretion into bile and for circulation to the kidneys.

The cost of detoxification is high; reactive intermediates and free radicals are generated and anti-oxidants like GSH are consumed. Any compromise of intestinal barrier function increases the production of oxygen radicals and carcinogens by the liver's cytochrome P-450 mixed function oxidase system.

A myriad of antigens

The intestinal mucosa is constantly exposed to a myriad of antigens, including bacteria and bacterial products (lipopolysaccharide (LPS), peptidoglycans), viruses, parasites and dietary antigens. The host has evolved sophisticated mechanisms to maintain homeostasis in the face of such a hostile environment. First and foremost, the intestinal luminal contents are isolated from the host by a single layer of cells, the intestinal epithelial cells (IECs).

These cells form a tight barrier that prevents potential toxic luminal products from breaching the mucosal layer and activating the underlying resident immune cells and/or gaining access to the systemic blood circulation.

In addition, an array of regulatory mechanisms such as the production of anti-inflammatory molecules (transforming growth factor (TGF)-β and interleukin (IL)-10), immunoglobulin A (IgA) synthesised by immune cells and various mucins produced by IECs participate in the maintenance of host homeostasis.

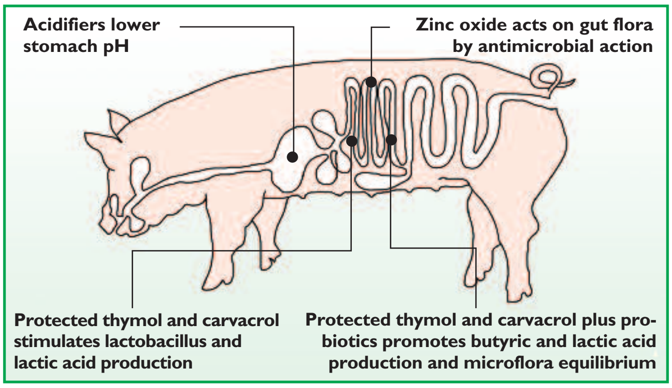

Fig. 1. Positioning of different feed additives (classical and alternatives) in the pig (Kamel and Gasa, 2007). Proposed sites of action of zinc oxide, acidifiers, prebiotics, probiotics and plant extracts in the intestine of the post-weaning pig.

Among the immune cells present in the intestinal mucosa, dendritic cells (DCs) play a pivotal role in sampling enteric antigens and presenting various microbial antigens to T lymphocytes, which then mature into either effector and/or regulatory cells. DCs are at the interface of innate and adaptive immunity and are active participants in intestinal homeostasis.

Used for decades

In use for many decades for their colourant properties for eggs, meat and skin in broilers, phytomolecules derived from yellow (Tagetes spp.) and red (Capsicum spp.) pigmented plants have drawn particular attention to their potential to support the gut through their antioxidant and immune activities during stress.

The ability of carotenoids to interact with free radicals have been the focus of several studies. Chew and Park (2004) reviewed the actions of pigment active molecules on DCs and other intestinal targets, channelling and modulating programmed immune responses.

Pigment derived flavonoids and other active substances are the focus of a European Framework grant in order to determine their roles in the immunity/ tolerance decision of the gut and how that affects the transition during the weaning period in pigs.

Plant extracts as triggers

The growth and hygiene benefits found from feeding antibiotics has been achieved through many different effects on the pig's gut. They are seen particularly during weaning, when the young pig's primitive gut tries to cope with the switch from simple, milk based to more complex, solid, vegetable based diets.

This period is often characterised by a gut flora dysbiosis. Dysbiosis is a term that is used routinely by nutritionists and veterinarians alike.

It refers to an abnormal, or disordered gut flora colonisation in the lower gut. Indeed, it not only relates to the relative populations of normal (and pathogenic) bacteria, but also to the potential of these to attach, colonise and enter into the mucosal barrier.

One such example lies in the disruption of the normal gut flora of young animals from predominately Gram positive organisms to a Gram negative, coliform flora (such as E. coli).

With the proliferation of E. coli, coliform endotoxin absorption ensues with malabsorption, as there is a direct injury to the normal mechanisms of intestinal homeostasis and metabolism.

Furthermore, gut flora dysbiosis is often a predisposing factor to other disease syndromes occasionally seen in the field such as necrotic enteritis in various post weaning pigs and poultry and others which permit the proliferation of more harmful, opportunistic organisms.

In swine waste studies little lactic acid (<5mM) accumulated in untreated swine waste incubations. However, when thymol or carvacrol are added, lactate accumulates to more than 30mM, with the greatest response from a 1:1 combination of thymol and carvacrol.

This indicates that these antimicrobial agents either inhibit a group of micro-organisms whereby the lactate producing micro-organisms are able to compete and produce lactate, or these agents suppress micro-organisms that utilise lactate. This supports later work which found that lactobacilli increased in the jejunum of piglets fed 1:1 thymol and carvacrol.

Based on this work the use of a three tiered approach including acidifiers, MOS or FOS and protected thymol and carvacrol produces a synergy of mode of action as a replacement for zinc oxide.

The action of acidifiers in the stomach and proximal intestine, in combination with protected thymol and carvacrol together with MOS and/or FOS reaching the lower gut, provides a total intestinal tract protection system for the young animal (Fig. 1).

Table 2. Means of three trials from 28-70 days of age. Treatments were: Zn oxide group – control diet (based on 44.5% maize, 14% barley, 17.5% soybean meal supplemented with acidifier blend at 3kg/ tonne and zinc oxide at 3,000g/tonne) versus control diet supplemented with acidifier at 3kg/tonne, protected thymol and carvacrol at 30g/tonne and fructo-oligo-saccharide at 1kg/tonne (Private Farm 2007).

Treatment	Zinc oxide	Acidifier + yeast cell wall + protected thymol & carvacrol
Starting liveweight at 28 days of age (kg/pig)	8.9	9.1
Ending liveweight at 70 days of age (kg/pig)	27.7	28.5
Daily weight gain (g/d)	447^{B}	462^{A}
Total feed intake (g/day)	955	952
Feed conversion ratio (g/g)	1.78^{B}	1.72^{A}
Mortality (n=)	4	5

A,B Means within a row with different superscripts differ significantly ($P \leq 0.10$).

Numerous trials

This is supported by a battery of consecutive trials with weaned pigs at a private European test farm using an acidifier with zinc oxide to reduce post weaning gut disturbances and even mortality in piglets.

Comparable results were measured with regard to feed intake, liveweight gain and feed conversion, with tendencies for improvement from using the three step strategy of acidifier + MOS/FOS + protected thymol and carvacrol in place of a conventional acidifier plus zinc oxide (Table 2). Moreover, a deeper look into the raw data indicated that the experimental groups on the three step strategy had more homogeneity, with fewer outlying animals.

It would be a valuable effect for units practising all-in/all-out production and being penalised if groups sent for slaughter lack uniformity.

Field trials in several European countries continue to confirm early observations that a research driven feed strategy based on solid science and proven micro-additives can provide a way of replacing age old remedies. In this particular case, plant extracts may provide several solutions to improve animal performance and give a better environment. ■