Do we have the tools to reduce antibiotics in swine production?

The global swine industry is going through unprecedented challenges. On the one hand, the threat of the African Swine Fever virus is global, despite the fact it hasn't arrived in all markets. The virus is today alive among the wild boars in the Polish and Belgian forests. Every day it keeps gaining a few more meters to the border, threatening the German swine industry, one of the largest in the European Union.

If this happens, we might be seeing important changes to the pork supply chain on the meat market worldwide – in Europe in addition to current issues in the USA meat plants. The profitability of swine businesses depends in many ways on the export capacity of large corporations based in Germany, Spain, Denmark, etc.

On the other hand, the presence of COVID-19 in most countries is changing human behavior, meat consumption at home, and the way we look at the future. Perhaps a virus overload via the news, some "fake news" conveying wrong messages on what's coming, and suddenly we feel the future will never be the same.

The future of the swine industry

At least for the swine industry, the future will indeed never be exactly the same. We will be facing different challenges. Some of these will be structural, such as the issue of decreased manpower and how to substitute manpower by machines, through the implementation of Precision Livestock Farming, for instance.

We are also facing important health challenges to our animals: not just ASF, but also new and more aggressive PRRS strains, among other pathogens. Our sows´ production capacity is increasing annually, yet in some cases 25% of the new-born piglets are lost from birth to market. Increasingly, we may start to see increased levels of mortality not only in the nursery but in fattening pigs and sows as well.

It is becoming clearer all the time: the future of the global swine industry lies in producing more pigs with reduced antibiotics. To stay the course, we need to take further action and implement corrective measures.

Why we should remove antibiotics in pig production

Pressure from stakeholders and regulators

There is, and there will be, increasing pressure from many stakeholders worldwide to work toward pig production with reduced or no antibiotics. Meat suppliers, slaughterhouses and processors, governments at different levels, and, of course, the European Union – all are demanding reductions in the level of antibiotics in livestock production.

There is also an increasing awareness at the global societal level regarding antimicrobial resistance related to antibiotic usage in farming production. Consumer pressure will grow exponentially as the terrible COVID-19 experience will be “digested” by the global population.

Pressure to accede to the pork market

There is yet another important reason to start working in that direction: the global swine meat market. Today, China’s pork meat shortage is opening the market. Now any producer could potentially sell meat, either to China or to any other country. We are starting to see moves from companies in the USA or Brazil banning the use of Ractopamine in their operations because they want to get access to the ractopamine-free market (Europe & Asia, over 70% of the global population).

According to M. Pierdon (AASV 2020 Proceedings), there will be two types of markets: the “Niche ABFree” and the “Commodity ABFree”. Companies will have to analyse what their future is on the meat market. Not all the producers may be willing to enter this new phase, but for sure many will try.

Strategies for antibiotic reduction

In Europe, the time has arrived. Zinc oxide will be banned in June 2021 and there is now more than a trend in production with less or no antibiotic use. In some cases, there is a need; in others, this is simply profitable.

Challenges to antibiotic reduction

Producing pigs completely without antibiotics is not easy, and not affordable for all. Initially we may have to give up some performance parameters in order to achieve the balance between what we want and what we can achieve in animal performance. But the time will arrive when these two objectives will converge; there is no alternative.

To that end, we will have to include in our pig production strategy all the available tools and technologies: genetic selection, immunization against some key pathogens, environmental control (mandatory but often forgotten), early detection of diseases, etc.

In this new era we are entering, nutrition and feed additives will play a key role. It will be crucial to find solutions targeting the microbiome's stabilization and diversification, creating and maintaining healthy farms and achieving all the performance parameters.

Do we have the tools for antibiotic reduction?

Even today there are companies able to produce completely antibiotic-free pigs – proof that **yes, the tools are already in place**.

Nevertheless, for most producers, the answer to – **Can we produce without antibiotics?** is most likely "probably not". This will require a holistic approach, given the specific case of piglets.

The microbiome of the piglet is strongly influenced by birth and the subsequent weeks. What, then, are the elements that will be part of this new game that comprises a new approach?

- The colostrum intake & the management of the piglets
- Antibiotic usage and its influence on the gut
- The piglets' microbiome and its evolution during the periweaning period
- The weaning process, appetite, and water intake
- Zinc oxide removal and its influence on the microbiome
- The immune system and the relationship with the GIT status
- Inflammation and its modulation at the gut level
- The health status and the effect on the concomitant infections: which ones are key and which ones are secondary pathogens
- The all-important biosecurity, management, and hygiene

To summarize: there is no one tool, but rather a **holistic approach** to face this new challenge that the swine industry is facing nowadays. The answer is not a silver bullet, but a journey that we all must undertake.

By Rafa Pedrazuela DVM, MBA – Available in Spanish here.
Global Technical Manager Swine, EW Nutrition

Respiratory Challenges: Breathing Space for Antibiotic Reduction?

Sub-therapeutic doses of antibiotic growth promoters (AGPs) were used for more than 50 years in poultry production to achieve performance targets – until growing concerns arose regarding antibiotic resistance (Kabir, 2009) and decreasing efficacy of antibiotics for medical purposes (Dibner & Richards, 2005).

Isolates of ESBL-producing *E.coli* from animals, farmworkers, and the environment were found to have identical multidrug resistance patterns (A. Nuangmek et al., 2018). There is also evidence that AMR strains of microorganisms spread from farm animal to animal workers and beyond. Global AMR fatalities are increasing and might reach 10 million by 2050 (Mulders et al., 2010, Trung et al., 2017, Huijbers et al., 2014).

In light of this, certain AGPs have already been banned, and there is a strong possibility of future restrictions on their use worldwide. Bans are effective: the MARAN report 2018 shows that lower antibiotics usage following the EU ban on AGPs has reduced resistant *E.coli* in broilers. Another positive consideration is the market opportunities that exist for antibiotic residue-free food.

However, the key element that poultry producers need to get right for antibiotic reduction to be successful is respiratory health management. This article looks at why respiratory health is a particular challenge – and how phytogenic solutions can help.

A closer look at the chickens' respiratory system

The respiratory tract is equipped with a functional mucociliary apparatus consisting of a protective mucous layer, airway surface liquid layer, and cilia on the surface of the ciliated cells. This apparatus produces mucus, which traps the inhaled particles and pathogens and propels them out of the airways. This mechanism, called the mucociliary clearance, is the primary innate defense mechanism of the respiratory system.

High stocking density combined with stressful environmental factors can negatively influence birds' immune systems (Heckert et al., 2002; Muniz et al., 2006), making them more susceptible to respiratory disease. When a bird suffers from respiratory disease, which is nowadays usually complicated by a co-infection or secondary bacterial infection, there is an excess production of mucus that results in ciliostasis and, therefore, in an impaired mucociliary clearance. The excess mucus in the tract obstructs the airways by forming plagues and plugs, resulting in dyspnea (hypoxia) and allowing the invasive bacteria to adhere and colonize the respiratory system.

The build-up of mucus in the respiratory tract severely reduces oxygen intake, causing breathlessness, reduced feed intake, and a drop in the birds' energy levels, which negatively impacts weight gain and egg production. Respiratory problems can result from infection with bacteria, viruses, and fungi, or exposure to allergens. The resultant irritation and inflammation of the respiratory tract leads to sneezing, wheezing, and coughing – and, therefore, the infection rapidly spreads within the flock.

Relatively high stocking density is the norm in poultry production

Low or no antibiotics: how to

manage respiratory disease?

Unsurprisingly, respiratory diseases in poultry are a major cause of mortality and economic loss in the poultry industry. For Complicated Chronic Respiratory Disease (CCRD), for instance, although the clinical manifestations are usually slow to develop, *Mycoplasma gallisepticum* (MG), in combination with *E. coli*, can cause severe airsacculitis. Beside feed and egg production reduction, these problems are of high economic significance since respiratory tract lesions can cause high morbidity, high mortality, and significant carcass condemnation and downgrading.

Producers need to pre-empt the spread of respiratory pathogens, react quickly to alleviate respiratory distress and maintain the mucociliary apparatus' functionality. Traditionally, treatment options are based on antiviral, anti-inflammatory, and antibiotic drugs. Can the poultry industry limit losses from respiratory infections without excessive recourse to antibiotics?

Indeed, a sudden reduction in antibiotic usage comes with a risk of impaired performance, increased mortality, and impaired animal health and welfare. The impact has been quantified as a 5% loss in broiler meat production per sq. meter (Gaucher et al., 2015). Effective antibiotics reduction requires a combination of innovative products and suitable consultancy services to manage poultry gut health, nutrition, flock management, biosecurity, and, particularly, respiratory health.

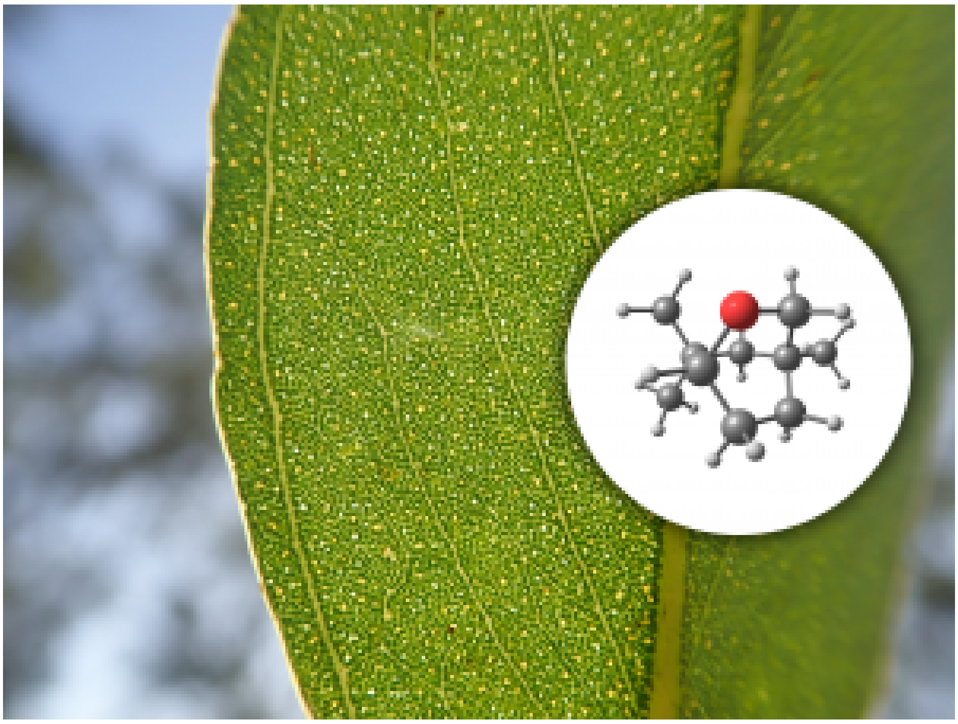
Non-antibiotic alternatives to control diseases and promote broiler growth, such as organic acids (Vieira et al., 2008), probiotics (Mountzouris et al., 2010), prebiotics (Patterson & Burkholder, 2003), and essential oils (Basmacioğlu Malayoğlu et al., 2010) have been the subject of much research in recent years.

Phytogenic solutions: proven efficacy

Essential oils, which are extracted from plant parts, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits, and roots, have a particularly well-established track record of medicinal applications. Efforts have centered on phytomolecules, the biologically active secondary metabolites that account for the properties of essential oils (Hernández et al., 2004; Jafari et al., 2011).

Studying these properties is challenging: essential oils are very complex natural mixtures of compounds whose chemical compositions and concentrations are variable. For example, the concentrations of the two predominant phytogenic components of thyme essential oils, thymol and carvacrol, have been reported to range from as low as 3% to 60% of the whole essential oil (Lawrence and Reynolds, 1984).

Another well-researched example is eucalyptus oil. The essential oils of eucalyptus species show antibacterial, anti-inflammatory, diaphoretic, antiseptic, analgesic effects (Cimanga et al., 2002) and antioxidant properties (Lee and Shibamoto, 2001; Damjanović Vratnica et al., 2011). The oils are mainly composed of terpenes and terpene derivatives in addition to some other non-terpene components (Edris, 2007). The principal constituent found in eucalyptus is 1,8-cineole (eucalyptol); however, other chemotypes such as α-phellandrene, ρ-cymene, γ-terpinene, ethanone, and spathulenol, among others, have been documented (Akin et al., 2010).



Close-up of eucalyptus leaf oil glands and
the molecular structure of eucalyptol $C_{10}H_{18}O$ (red = oxygen; dark grey = carbon; light grey = hydrogen)

Antimicrobial activity

In modern intensive broiler production, bacterial diseases such as salmonellosis, colibacillosis, mycoplasmosis, or clostridia pose serious problems for the respiratory system and other areas. Analyses of the antibacterial properties of essential oils have been carried out by multiple research units (Ouwehand et al., 2010; Pilau et al., 2011; Solorzano- Santos and Miranda-Novales, 2012; Mahboubi et al., 2013; Nazzaro et al., 2013; Petrova et al., 2013).

Phenols, alcohols, ketones, and aldehydes are clearly associated with antibacterial activity; the exact mechanisms of action, however, are not yet fully understood (Nazzaro et al., 2013). Essential oils' antimicrobial activity is not attributable to a unique mechanism but instead results from a cascade of reactions involving the entire bacterial cell (Nazzaro et al., 2013). However, it is accepted that antimicrobial activity depends on the lipophilic character of the components.

The components permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane-bound electron flow and thus the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP (adenosine triphosphate) pool. High concentrations may also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Nazzaro et al., 2013; Gopi et al., 2014).

According to current knowledge, lavender, thyme, and eucalyptus oil, as well as the phytomolecules they contain, show enhanced effects when combined with other essential oils or synthetic antibiotics (Sadlon and Lamson, 2010; Bassole and Juliani, 2012; Sienkiewicz, 2012; de Rapper et al., 2013; Zengin and Baysal, 2014).

Minimum inhibitory concentration (MIC) of some essential oil components against microorganisms *in vitro*

Immune system boost I: improved production of antibodies

Some essential oils were found to influence the avian immune system positively, since they promote the production of immunoglobulins, enhance the lymphocytic activity, and boost interferon-γ release (Awaad et al., 2010; Faramarzi et al., 2013; Gopi et al., 2014; Krishan and Narang, 2014). Placha et al. (2014) showed that the addition of 0.5g of thyme oil per kg of feed significantly increased IgA levels.

Awaad et al. (2010) experimented on birds vaccinated with the inactivated H5N2 avian influenza vaccine. The experiment revealed that adding eucalyptus and peppermint essential oils to the water at a rate of 0.25 ml per liter resulted in an enhanced cell-mediated and humoral immune response.

Saleh et al. (2014), who applied thyme and ginger oils in quantities of 100mg and 200mg per kg of feed, respectively, observed an improvement in chickens' immunological blood profile through increased antibody production. Rehman et al. (2013) stated that the use of herbal products containing eucalyptus oil and menthol in broilers showed consistently higher antibody titers against NDV (Newcastle disease virus), compared to untreated broilers.

Immune system boost II: better vaccine responses and anti-inflammatory effects

Essential oils are also used as immunomodulators during periods when birds are exposed to stress, acting protectively and regeneratively. Importantly, the oils alleviate the stress caused by vaccination (Barbour et al., 2011; Faramarzi et al., 2013; Gopi et al., 2014). The study by Kongkathip et al. (2010) confirmed the antiviral activity of turmeric essential oil.

In recent years studies have been carried out on the use of essential oils in conjunction with vaccination programs, including those against infectious bronchitis (IB), Newcastle disease, and Gumboro disease. The results of the experiments show that essential oils promote the production of antibodies, thus enhancing the efficacy of vaccination (Awaad et al., 2010; Barbour et al., 2010; Barbour et al., 2011; Faramarzi et al., 2013).

Essential oils contain compounds that are known to possess strong anti-inflammatory properties, mainly terpenoids, and flavonoids, which suppress the metabolism of inflammatory prostaglandins (Krishan and Narang, 2014). Also, other compounds found in essential oils have anti-inflammatory, pain-relieving, or edema-reducing properties, for example, linalool from lavender oil, or 1,8-cineole, the main component of eucalyptus oil (Peana et al., 2003).

Immune system boost III: antioxidant effects and radical scavenging

An imbalance in the rate of production of free radicals or removal by the antioxidant defense mechanisms leads to a phenomenon referred to as oxidative stress. A mixture of Oregano (carvacrol, cinnamaldehyde, and capsicum oleoresin) was found to beneficially affect the intestinal microflora, absorption, digestion, weight gain and also to have an antioxidant effect on chickens (Bassett, 2000).

Zeng et al. (2015) indicated the positive effect of essential oils on the production of digestive secretions and nutrient absorption. They reduce pathogenic stress in the gut, exert antioxidant properties, and reinforce the animal's immune status.

Inside the cell, essential oils can serve as powerful scavenger preventing mutations and oxidation (Bakkali et al., 2008). Studies have demonstrated the concentration-dependent free radical scavenging ability of oils from eucalyptus species (Kaur et al., 2010; Marzoug et al., 2011; Olayinka et al., 2012). Some authors attribute the strong antioxidant capacity of essential oils to their phenolic constituents and synergistic effect between tannins, rutin, thymol, and carvacrol, and probably 1, 8-cineole. Moderate DPPH radical scavenging activity reported by Edris(2007), El-Moein et al. (2012), and Kaur et al. (2011).

Vázquez et al. (2012) have demonstrated the potential of the phenolic compounds in eucalyptus bark as a source of antioxidant compounds. The study showed that eucalyptus had ferric reducing antioxidant power in the ranges 0.91 to 2.58 g gallic acid equivalent (GAE) per 100 g oven-dried bark and 4.70 to 11.96 mmol ascorbic acid equivalent (AAE) per 100 g oven-dried bark, respectively (see also Shahwar et al., 2012). Moreover, Eyles et al. (2004) were able to show superoxide dismutase (SOD)-like activity for different compounds and fractions isolated from wood extracts.

Last but not least: positive effects on the respiratory system

In poultry production houses, especially in summer, high temperatures and low humidity increase the amount of air dust. Under such conditions, respiratory tract disorders in broiler chickens, including the deposition of particulates, become more frequent and more severe.

Clinical signs of respiratory disease in chickens include coughing, sneezing, and rales

Thyme oil, thanks to the phytomolecules thymol and carvacrol, supports the treatment of respiratory disorders. These substances smooth tightened muscles and stimulate the respiratory system. An additional advantage lies in their expectorant and spasmolytic properties (Edris, 2007).

These properties are also seen in essential oils such as eucalyptus and peppermint, which contain eucalyptol and menthol. They thin out the mucus and facilitate its removal from the airways. As a result, the airways are cleared and breathing during inflammation becomes easier (Durmic and Blache, 2012).

Another positive effect of the terpenoid compounds used in commercial preparations for poultry is that they disinfect the bronchi, preventing respiratory infections (Awaad et al., 2010; Barbour et al., 2011; Mahboubi et al., 2013). Barbour and Danker (2005) reported that the essential oils of eucalyptus and peppermint improved the homogeneity of immune responses and performance in MG/H9N2-infected broilers.

Grippozon: the phytogenic solution for respiratory health

Grippozon is a liquid composition with a high content of essential oils, which are combined to systematically prevent and ease respiratory diseases. The formulation is derived from the research on essential oils' effectiveness against respiratory pathogens that are common in animal farming. Grippozon exhibits a synergistic action of all its components to optimally support animal health. It contains a high concentration of active components; both their quantity and quality are guaranteed to deliver results.

Application of Grippozon

Grippozon application can be flexibly adapted to most common housing systems. It is fully water-soluble for use in the drinking line and it is also possible to nebulize a diluted solution in air.

The dose recommendation in drinking water usually amounts to 100ml to 200ml per 1000 liters of drinking water (Grippozon administration has not been reported to affect water consumption). The active substances in Grippozon adhere to mouth mucosa and become volatile in the breathing air later on. Therefore Grippozon can enter the respiratory system indirectly as well. The volatile compounds also spread into the whole barn air and, thus, indirectly via breathing into the respiratory system (and farmers notice the smell of essential oils when Grippozon is applied through in the waterline)

Grippozon can also be used as a spray at a rate of 200ml/10 liters of water for 2000 birds, twice daily on 2-3 days a week. This produces a very effective nebulization effect and offers faster respiratory relief to birds.

Grippozon is an impactful tool for managing respiratory problems. Thanks to its effective mucolytic and relaxant activity, Grippozon gives symptomatic relief to the birds during high-stress periods of respiratory diseases. Mucus in the trachea works as media for the proliferation of bacteria and viruses, so by thinning the mucus, Grippozon slows down the proliferation of bacteria and the spread of disease. Grippozon helps in improving air quality and air intake. It can also be used to stimulate the immune response during vaccination.

Authors:
Ruturaj Patil – Product Manager Phytogenic Liquids
Kowsigaraj Palanisamy – Global Validation Trial Manager

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5 principles to consider when designing biosecurity programmes

Biosecurity is the foundation for all disease prevention programs and all the more important in antibiotic reduction scenarios. It includes the combination of all measures taken to reduce the risk of introduction and spread of diseases and is based on the prevention of and protection against infectious agents. Its fundament is the knowledge of disease transmission processes.

Although biosecurity is considered the cheapest and most effective intervention in antibiotic reduction programmes, compliance is often low and difficult.

The application of consistently high standards of biosecurity can substantially contribute to the reduction of antimicrobial resistance, not only by preventing the introduction of resistance genes into the farm but also by lowering the need to use antimicrobials.

Lower use of antimicrobials with higher biosecurity

Studies and assessments such as those done by (Laanen, *et al*., 2013), (Gelaude, *et al*., 2014), (Postma, *et al*., 2016), (Collineau, *et al*., 2017) and (Collineau, *et al*., 2017a) relate a high farm biosecurity or improvements in biosecurity with lower antimicrobial use. Laanen, Postma, and Collineau studied the profile of swine farmers in different European countries, finding a relation between a high level of internal biosecurity, efficient control of infectious diseases, and a reduced need for antimicrobials.

Others such as Gelaude and Collineau studied the effect of interventions. The former examined Belgian broiler farms, finding a reduction of antimicrobial use by almost 30% when biosecurity and other farm issues were improved within a year. The latter studied swine farms located in Belgium, France, Germany and Sweden, in which antimicrobial use was also reduced in 47% across all farms and observed that farms with the higher biosecurity compliance and who also took a holistic approach, making other changes (e.g. management and nutrition), achieved a higher reduction in antimicrobial use.

Biosecurity interventions pay off

Of course, the interventions necessary to achieve an increased level of biosecurity carry some costs. However, the interventions, especially if taken with other measures such as improved management of new-born animals and nutritional improvements, also improve productivity. The same studies which report that biosecurity improvements decrease antimicrobial use also report an improvement in animal performance. In the case of broilers, Laanen (2013) found a reduction of 0.5 percentual points in mortality and one point in FCR; and Collineau (2017) obtained an improvement during both the pre-weaning and the fattening period of 0.7 and 0.9 percentual points, respectively.

Implementation, application and execution

Although biosecurity is considered the cheapest and most effective intervention in antibiotic reduction programmes, compliance is often low and difficult. The implementation, application, and execution of any biosecurity programme involve adopting a set of attitudes and behaviours to reduce the risk of entrance and spread of disease in all activities involving animal production or animal care. Measures should not be constraints but part of a process aimed at improving the health of animals and people, and a piece of the holistic approach to reduce antibiotics and improve performance.

Designing effective biosecurity programmes: Consider these 5 principles

When designing or evaluating biosecurity programmes, we can identify 5 principles that need to be applied. These principles set the ground for considering and evaluating biosecurity interventions:

1. Separation: ***Know your enemy, but don't keep it close***

It is vital to have a good separation between high and low-risk animals or areas on the farm, as well as dirty (general traffic) and clean (internal movements) areas on the farm. This avoids not only the entrance but the spread of disease, as possible sources of infection (e.g. wild birds) cannot reach the sensitive population.

2. Reduction: ***Weaken your enemy, so it doesn't spread***

The goal of the biosecurity measures is to keep infection pressure beneath the level which allows the natural immunity of the animals to cope with the infections, lowering the pressure of infection e.g. by an effective cleaning and disinfection programme, by the reduction of the stocking density, and by changing footwear when entering a production house.

3. Focus: *Hunt the elephant in the room, shoo the butterflies*

In each production unit, some pathogens can be identified as of high economic importance. For each of these, it is necessary to understand the likely routes of introduction into a farm and how it can spread within it. Taking into account that not all disease transmission routes are equally important, the design of the biosecurity programme should focus first on high-risk transmission routes, and only subsequently on the lower-risk transmission routes.

4. Repetition: ***Increasing the probability of infection***

In addition to the probability of pathogen transmission via the different transmission routes, the frequency of occurrence of the transmission route is also highly significant when evaluating a risk (Alarcon, *et al.,* 2013). When designing biosecurity programmes, risky actions such as veterinary visits, if repeated regularly must be considered with a higher risk.

5. Scaling: ***In the multitude, it is easy to disguise***

The risks related to disease introduction and spread are much more important in big; more animals may be infected and maintain the infection cycle, also large flocks/herds increase the infection pressure and increase the risk by contact with external elements such as feed, visitors, etc.

Can we still improve our biosecurity?

Almost 100% of poultry and swine operations already have a nominal biosecurity programme, but not in all cases is it effective or completely effective. BioCheck UGent, a standardised biosecurity questionnaire applied worldwide, shows an average of 65% and 68% of conformity, from more than 1000 broiler and 2000 swine farms between respectively; opportunities to improve can be found in farms globally, and they pay off.

The bottom line

Biosecurity is necessary for disease prevention in any profitable animal production system. To make effective plans, these 5 principles should be applied to choose the right interventions that prevent the entrance and spread of disease. However, maintaining a successful production unit requires a holistic approach in which other aspects of biosecurity need to also be taken seriously, as well as actions to improve in other areas such as management, health and nutrition.

Authors: Marisabel Caballero, Global Technical Manager Poultry – EW Nutrition, and Fellipe Freitas Barbosa, Team Lead Global Technical Management Swine – EW Nutrition

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Understanding and managing Strep suis in swine: The essentials

***Strep suis* causes vast losses in pig production and threatens human health, too. We still rely on antibiotics to control it – but we will have to change tactics to contain antimicrobial resistance.**

Streptococcus suis is one of the most economically harmful pathogens for the global swine industry. When I started working in pig production 25 years ago, *S. suis* was already a problem on practically all the farms that I visited. Back then, our understanding of the pathogen and hence our control strategies were rudimentary: in farrowing rooms, we cut piglets' teeth, used gentian violet spray on their navels, and sometimes applied penicillin lyophilized with iron. For the nursery phase, we only had penicillin or phenoxymethylpenicillin at our disposal – until the first amoxicillin-based premixes arrived, which turned out to be highly effective.

To this day, we control *S. suis* mainly through oral beta-lactam antibiotics (in feed or water) or injectable solutions, administered to piglets at an early age. However, pig production has evolved dramatically over the past decades, and so has the scientific research on this complex pathogen. Crucially, we now know that the excessive use of antibiotics contributes to the development of antimicrobial resistance.

Recent Australian research has discovered *S. suis* strains (both in humans and pigs) with a high degree of resistance to macrolides or tetracyclines, strains with intermediate sensitivity to Florfenicol, and others that are developing resistance to penicillin G. Additionally, we now know that *S. suis* is a zoonotic bacteria that affects not only at-risk farm or slaughterhouse personnel: *S. suis* is among the leading causes of death from meningitis in countries such as Thailand, China or Vietnam. In light of these threats to human health, we in the swine industry more than ever have a duty to help control this pathogen.

This article first reviews our current state of knowledge about the epidemiology and pathogenesis of *Strep suis*; it then lays out virulence factors and the role of coinfections. The second part considers the dimensions of a holistic approach to *S. suis* prevention and control and highlights the central role of intestinal health management.

What we know about *S. suis* epidemiology and pathogenesis

Practically all farms worldwide have carrier animals, but the percentage of animals colonized "intra-farm" varies between 40 and 80%, depending on several factors such as environmental conditions, hygiene measures, and the virulence of the *S. suis* strains involved.

How *S. suis* strains are classified

S. suis strains were once classified into 35 serotypes, according to their different *capsular polysaccharides*(*CPS*), *the*outermost layer of *the* bacterial cell. Due to phylogenic and genomic sequencing, some of the old serotypes (20, 22, 26, 32, 33, and 34) are now reclassified, either in other bacterial genera or in other *Streptococcus* species. This has reduced the total to 29 *S. suis* serotypes.

Globally, the prevalence of the disease varies between 3% and 30%. The main serotypes affecting pig population are type 2 (28%), 9 (20%), and 3 (16%); differences in the geographical distribution are shown in Figure 1.

Figure 1: Global distribution of S. suis serotypes
Based on different sources, incl. Goyette-Desjardins et al. (2014), Zimmermann et al. (2019), and Gebhart (2019)

In addition to the serotype classification based on CPS antigens, *S. suis* has also been genetically differentiated into "sequence types" using the MLST (Multi Locus Sequence Typing) technique. The distribution of both porcine and human sequence types is detailed in Figure 2.

Figure 2: S. suis sequence types and their worldwide distribution

How *S. suis* is transmitted in swine

The main transmission routes are, firstly, the vertical sow-piglet route; the mucosa of the vagina is the first point of contamination. In the farrowing room, respiratory transmission from the sow to the piglets takes place. Horizontal transmission between piglets has also been proven to occur, especially during outbreaks in the post-weaning phase. This form of transmission happens through aerosols, feces, and saliva.

While in humans, the possibility of infection via the digestive tract has been confirmed, there are discussions about this route for swine. De Greeff et al. (2020) argue, based on *in vitro* and *in vivo* data, that infection through the digestive tract is associated with specific serotypes. Serotype 9, for example, would have a greater capacity for colonizing the gastrointestinal tract, and from there, the bacteria's translocation takes place. The same authors point out that, in Western Europe, *S. suis* serotype 9 has become one of the most prevalent serotypes in recent years.

How *S. suis* colonization occurs

Although there are still unknown mechanisms in the pathogenesis of the disease, it can be schematically summarized how colonization occurs (Figure 3). From the different infection routes, the pathogen always passes through the mucosa. When *S. suis* enter the bloodstream, it can lead to a systemic infection, ending in septicemia, meningitis, endocarditis, or pneumonia, or a local infection at the joints level, causing arthritis.

According to Haas and Grenier (2018), different pathogenicity factors intervene in each of the processes. The CPS, for example, are relevant during colonization and the initial progression (indicated by black arrows). Microvesicles released by *S. suis* cell membranes are more involved in the passage to the bloodstream or, for example, the progression towards local or systemic infection (indicated by white arrows).

Figure 3: Pathogenesis of S. suis infection
Source: based on Haas and Grenier (2018)

Depending on the host and the immune response, the well-known clinical signs of the disease will occur. Although they may begin in the lactation phase, the highest prevalence of meningitis (the main clinical symptom) usually occurs between the 5th and the 10th week of life, that is, between two and three weeks after weaning.

How to diagnose *S. suis* infection

Diagnosing *S. suis* is relatively simple at a clinical level; however, we need to know how to differentiate it from *G. parasuis* in the case of animals with nervous symptoms. We also need to distinguish *S. suis* from other pathogens responsible for producing arthritis, such as *M. hyosynoviae* or the fibrin-producing agent *M. hyorhinis*.

Laboratory techniques are developing on two fronts. Among molecular techniques, multilocus sequence typing (MLST) is considered the gold standard for serotyping. It is still costly and not yet practicable for large samples at the farm level. In contrast, several types of polymerase chain reaction (PCR) show greater practical applicability. Quantitative PCRs (qPCR) are used for the evaluation of bacterial load, and some PCRs are based on the identification of specific virulence genes.

Due to the relevance of *S. suis* for human health, more complex techniques are also available, such as the complete sequencing of the bacterial genome. This type of method aims to develop epidemiological analyzes together with the differentiation between virulent and non-virulent *S. suis* strains. Research is also underway in serology, particularly on evaluating maternal immunity and its interference with the piglet, as well as autogenous vaccines monitoring.

Why *S. suis* sometimes causes disease: Virulence factors and coinfections

Streptococcus suis is a pathobiont, i.e., a microorganism that belongs to the commensal flora of animals but generates disease under certain conditions. In their daily work on farms, clinical veterinarians, for instance, find that *S. suis* often colonizes the upper respiratory tract, nasal cavity, and tonsils without causing disease. *S. suis* pathogenicity is associated with an astounding range of different circumstances or triggering factors; some sources list more than 100 virulence factors. Several factors are considered essential in the development of pathogenesis; others, however, are the subject of ongoing research (cf. Xia et al., 2019, and Segura et al., 2017).

Critical virulence factors

- One of the most important proteins is the CPS that establishes serotypes. The CPS largely determines the bacteria's adhesion and colonization behavior. It can modify its thickness depending on the stage: it becomes thinner when adhering to the mucociliary apparatus and thicker when circulating through the bloodstream, protecting the bacteria against possible attacks by immune system cells.
- Likewise, *suis* has an adhesin known as Protection Factor H (FHB) that protects it from phagocytosis by macrophages and can also interfere with the complement activation pathways of the immune system.
- Suilysin is one of the most critical *suis*' protein toxins. This toxin plays a fundamental role in the interaction with host cells (modulating them to facilitate invasion and replication within the host cells) as well as in the inflammatory response.
- *S. suis* is a mucosal pathogen and, hence, triggers a mucosal immunity response, mainly by immunoglobulins A (IgA). *S. suis* has developed proteases capable of destroying both IgA and IgG.
- Research is still in progress, but both *suis* serotype 2 and 9 encode the development of adhesion proteins that facilitate mucociliary colonization when salivary glycoproteins are present (these are called antigens 1 and 2).
- Other than Suilysin, two of the bacteria's protein components that have been studied in-depth to develop subunit vaccines are the MRP (Muramidase Release Protein) and EF (Extracellular Factor) protein. Whether the expression of these proteins is associated with virulence depends on the serotype.
- Recent research indicates that greater biofilm production capacity is associated with the more virulent *suis* strains. The production of biofilm is closely related to the production of fibrinogen, which allows the bacteria to develop resistance to the action of antimicrobials, to colonize tissues, to evade the immune system, etc.

Concomitant factors for *S. suis* infection

Even though *S. suis* is a primary pathogen that can cause disease by itself, many factors can exert a direct or indirect influence on whether or not and to which extent disease develops.

Veterinarians and producers are well aware of the influence of environmental and management factors such as temperature variations, poor ventilation together with poor air quality, irritants for the respiratory tract, as well as correct densities for animals' welfare. Occasionally, depending on the geographical location, *S. suis* can be considered as a seasonal pathogen, showing a higher prevalence during the coldest months of the year when ventilation is lower or not well-controlled.

At the level of the individual animal, concomitant pathogens, environmental changes, diet changes, previous pathologies, piglet handling problems, etc., all come into play. Younger piglets tend to be more susceptible because of the decrease in maternal immunity or insufficient colostrum intake; diarrhea during the lactation phase also increases disease vulnerability.

Recently, researchers have started to explore the hypothesis that a change in the digestive tract microbiome balance may favor a pathogenic trajectory. Some results indicate that changes in the microbiota around the moment of weaning could indeed trigger disease. I will return to the vital topic of the digestive tract in *S. suis* pathogenesis below.

The role of coinfections

The virulence of *S. suis* can increase in the presence of other pathogens, both viral and bacterial. Among the main viruses, key interactants are the PRRS virus, the influenza virus (SIV), as well as Porcine Circovirus (PCV) and Porcine Respiratory Coronavirus (PRCV). At the bacterial level, *Bordetella bronchiseptica* and *Glaesserella parasuis* have the most direct interaction with *S. suis* (Brockmeier, 2020).

There are several mechanisms by which coinfections might increase S. suis virulence: some of them (i.e., *B. bronchiseptica* and SIV) alter the epithelial barrier, facilitating the translocation of *S. suis*. Moreover, viruses such as PRRS either cause an alteration in the response of the immune system or destroy relevant immune system cells.

Valentin-Weigand et al. (2020) posit that the influenza virus increases the pathogenic capacity of *S. sui*s so that, for specific strains, the disease can develop even in the absence of the key virulence factor suilysin. This highlights the importance of controlling coinfections for successful *S. suis* management.

The five pillars of holistic *S. suis* management in swine

The challenge of managing this problematic pathogen with limited use of antibiotics prompts a review of all strategies within our reach. From birth to slaughterhouse, interventions must be coordinated and cannot work independently.

1. Biosecurity

The principles of biosecurity are easily understood. Yet, across different locations and production systems, farms struggle with consistently executing biosecurity protocols. For the moment, it appears unrealistic to avoid the introduction of new *S. suis* strains altogether. Also, complete eradication is not feasible with the currently available tools.

Genetic companies and research centers will likely continue to explore how to reduce bacterial colonization in animals, to produce piglets that have no or only minimal *S. suis* populations. Again, this option is not available for now.

At the farm level, the most promising and feasible approach is to reduce the risk of bacterial transmission, i.e., to optimize internal biosecurity. This extends to controlling both viral and bacterial coinfections. The two major viruses affecting the nursery stage are the PRRS virus and Swine Influenza virus. Bacteria that can contribute to the disintegration of the mucosa, both at the respiratory level and the digestive level, are Atrophic Rhinitis (progressive or not) and digestive pathogens such as *E. coli*, Rotavirus and *Eimeria suis*. All possible measures to reduce the prevalence and spread of these co-infectants must be executed to help control *S. suis*.

2. The pre-weaning period

We need to consider several elements in the first hours after birth that influence the spread of the bacteria in the farrowing rooms:

- How is the colostrum distribution between the litters and the subsequent distribution of the piglets carried out?
- How is the "processing" of the piglets carried out after farrowing: iron administration, wound management, and tail docking?
- Are we taking any measure to prevent iatrogenic transmission of pathogens through needle exchange?

Until today, it is common practice to administer systemic (in-feed) or local (vaginally applied) antibiotics during the pre-weaning phase, albeit with partial or inconsistent successes in terms of reducing infection pressure. Notably, during the pre-weaning phase, the development of the piglet's microbiota begins to take shape, and the systematic and prophylactic application of antibiotics in young animals can reduce bacterial diversity of the microbiome (Correa-Fiz et al., 2019). This, in turn, leads to a proliferation of bacteria with a pathogenic profile that could detrimentally influence subsequent pathology.

S. suis is an ultra-early colonizer; piglets can get infected at birth already

3. The post-weaning period

The post-weaning period undoubtedly constitutes the most critical stage of the piglets' first weeks of life. In addition to social and nutritional stress, piglets are exposed to new pathogens. While maternal immunity is decreasing, piglets have not developed innate immunity yet; they are now most susceptible to the horizontal transmission of diseases. Hence, *S. suis* prevention during this phase center on measures that improve piglet quality. Key parameters include:

- Do we have a correct and homogeneous weight/age ratio at weaning?
- What is the level of anorexia in piglets? Do we practice suitable corrective measures to encourage the consumption of post-weaning feed?
- How are we feeding them? What medications do they routinely receive?
- How are housing facilities set up concerning density, environment, and hygiene?

Again, gut health is critical: Ferrando and Schultsz (2016) suggest that the status of the piglet's weaning gastrointestinal tract centrally influences the subsequent development of the disease. Their research supports the idea that some specific *S. suis* serotypes can develop their pathogenesis from the digestive tract, just as in human medicine. While in humans, this digestive route is associated with the consumption of raw or insufficiently processed pork, in swine, the most susceptible moments are sudden changes in diet. The transition from milk to solid feed, in particular, leads to an increase in alpha-glucans that favor bacteria proliferation. Likewise, an increase in susceptibility occurs when the integrity of the intestinal wall is lost, for example, due to viral and bacterial coinfections.

4. Treatments and vaccination

Since weaning is such a difficult phase for the life of the piglet, it is a common practice on farms across the world to include one or several antibiotics in the post-weaning phase. Sometimes, when the legal framework allows, producers use a systematic antibiotic (i.e., beta-lactams or tetracyclines) and another one with a digestive profile (e.g., pharmacological doses of ZnO, trimethoprim, sulfa drugs and derivatives).

While antibiotics, for the most part, effectively prevent infection in the post-weaning phase, they can have adverse effects on the digestive tract. According to Zeineldin, Aldrige, and Lowe (2019), continued antibiotics use:

- might increase the susceptibility to other infections because of the imbalance of the microbiome,
- the immune system might be weakened, together with an alteration in metabolism,
- and it fosters a greater accumulation of bacteria that are resistant to antibiotics.

The effectiveness of curative antibiotics treatments varies considerably. In any case, early detection is critical; affected animals need to be isolated and provided with a comfortable environment. Therapeutic parenteral antibiotics are best combined with high-dose corticosteroids. Some sick animals are unable to stand or walk. As a complementary measure, it is recommended, where possible, to help them ingest some feed and water.

Much research attention is focused on finding suitable vaccines to control the disease. This is a challenging task: *S. suis* shows high genetic diversity, making the identification of common proteins difficult, and is protected against antibody binding by a sugar-based envelope. The research group around Mariela Segura and Marcelo Gottschalk, for example, is working on a subunit vaccine strategy that addresses both dimensions. Recently, Arenas et al. (2019) identified infection-site specific patterns of *S. suis* gene expression, which could serve as a target for future vaccines.

The arrival of a universal, affordable *S. suis* vaccine is still a distant hope, though. Inactivated vaccines generally offer low levels of antibodies at the mucosal level and would need some adjuvant to increase them. A multiple injection protocol will not work from a commercial and practical point of view. On the other hand, live attenuated vaccines risk re-developing virulence with potentially drastic effects on human health. To complicate the topic of vaccination further, there is a controversy regarding the time of application and what animals we should vaccinate – sows, piglets, both?

Today, though with variable results, the alternative to scarce commercial vaccines is autogenous vaccines. These are based on the suspected serotype(s) present on a particular farm. This strategy hinges on the difficult procedure of isolating the strain from the meninges, spleen, or joints of the animals. If this step is successful, a laboratory can then develop the autogenous vaccine. Immunization occurs mainly in piglets, but occasionally some sows are vaccinated during the lactation period.

5. Hygiene

Just as for any other pathogen, hygiene management is critical. The infection pressure can be lowered through simple steps, such as washing the breeders before they enter the farrowing room. It is, or it should be, standard practice to maximize hygiene in the processing of piglets, avoiding injuries or pinching of the gums during teeth cutting, as well as disinfecting the umbilical area.

We know that *S. suis* is usually very sensitive to most disinfectants, but that is can form a biofilm that allows it to withstand hostile conditions. Physical or chemical methods to eliminate biofilm-formation are thus vital for combatting *S. suis* effectively.

Figure 4: The 5 pillars of S. suis control and prevention

S. suis control and prevention: The future lies in the gut

There is no ideal solution for totally controlling *S. suis* yet: autogenous vaccines are only partially effective, and since we cannot continue to administer antibiotics systematically, it is necessary to look for alternatives. Pending the arrival of a universal vaccine, the most promising efforts focus on the gastrointestinal tract.

Microbiome balance to keep *S. suis* in check

The gastrointestinal tract is not only the site where nutrient absorption takes place. The gut is the largest immune system organ in the body and most exposed to different antigens; therefore, what happens at the digestive level has a considerable influence on the immune system, locally and systemically.

The microbiome can be defined as the set of autochthonous bacteria that reside in the digestive system of animals. This group of bacteria is continually evolving and changes at critical moments in the life of animals. Simply put, a healthy microbiome is one that has a high bacterial diversity in the digestive tract (alpha diversity). The diversity between animals, on the other hand, should be low (beta diversity). A healthy microbiota implies the absence of dysbiosis and pathogens. Finally, one wants to promote the presence of bacteria that can produce substances with a bactericidal effect, such as short-chain fatty acids or bacteriocins.

Can we influence the microbiome to have fewer *S. suis* problems? Research by Wells, Aragon, and Bessems (2019) compared microbiota samples of the palatine tonsils from healthy and infected animals. They found that animals that would later develop the disease showed less diversity and, in particular, a diminished presence of the genus *Moxarella*. Importantly, they found that these differences in the microbiome's composition of animals that later developed the disease were noticeable *before* weaning and at least two weeks before the outbreak occurred.

The same authors investigated in more depth, which bacteria in the microbiome were able to maintain homeostasis at the digestive level, finding that this was mostly the case for the genera *Actinobacillus, Streptocuccus*, and *Moraxella*. Moreover, they found that *Prevotellacea* and *Rhotia* produce antibacterial substances against *S. suis*.

Nutrition can impact the microbiome through targeted ingredients

We have to think about the microbiome of locations other than the digestive system as well. As we previously saw, the bacteria are transmitted through the mucosal route in the vagina, through the respiratory route, and there are recent studies that consider saliva as a leading source of infection in oral transmission.

This research contributes insights into how we might approach *S. suis* management through nutritional strategies. The question for nutritionists is, can you formulate feed that reduces the availability of *S. suis*' favorite nutrients? *S. suis* appears to develop best when the feed contains large quantities of carbohydrates or starches. Other nutritional factors include the feed's buffering capacity and the stomach pH of the piglets.

In times of antimicrobial resistance, additives are crucial for S. suis control and prevention

Gut health and nutrition approaches come together in the area of additives: targeted gut health-enhancing additives to feed or water will become a cornerstone of *S. suis* control. What we want to see in such products are molecules or substances that are capable of limiting, inhibiting, or slowing down the growth of *S. suis* by altering the membrane or interfering with the energy mechanisms of the bacteria.

There are already several products on the market with different active ingredients, such as phytomolecules, medium-chain fatty acids, organic acids, prebiotics, probiotics, etc. Soon, those products or combinations of them will be a part of our strategy for controlling this pathogen of such importance to our industry.

Author: Rafa Pedrazuela, Global Technical Manager Swine – EW Nutrition

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Challenging times for broilers? Phytomolecules, not antibiotics, are the answer

*by **Ajay Bhoyar**, Global Technical Manager, EW Nutrition*

Anyone working with today's fast-growing broiler chicken knows that it is a sensitive creature – and so is its gut health. Thanks to continuous improvements in terms of genetics and breeding, nutrition and feeding, as well as general management strategies, broiler production has tremendously upped performance and efficiency over the past decades. It is estimated that, between 1957 and 2005, the broiler growth rate increased by over 400%, while the feed conversion ratio dropped by 50%.

These impressive improvements, however, have come at the cost of intense pressure on the birds' digestive system, which needs to process large quantities of feed in little time. To achieve optimal growth, a broiler's gastrointestinal tract (GIT) needs to be in perfect health, all the time. Unsurprisingly, enteric diseases such as necrotic enteritis, which severely damages the intestinal mucosa, hamper the intestines' capacity to absorb nutrients and induce an inflammatory immune response.

The modern broiler's gut – a high-performing,

but sensitive system

However, in a system as high performing as the modern broiler's GIT, much less can lead to problems. From when they are day-old chicks up to slaughter, broilers go through several challenging phases during which they are more likely to show impaired gut functionality, e.g. after vaccinations or feed changes. Good management practices go a long way towards eliminating unnecessary stressors for the animals, but some challenging periods are unavoidable.

The transition from starter to grower diets is a classic situation when nutrients are very likely to not be well digested and build up in the gut, fueling the proliferation of harmful microbes. Immunosuppressive stress in combination with an immature intestinal microflora results in disturbances to the bacterial microbiota. At "best", this entails temporarily reduce nutrient absorption, in the worst case the birds will suffer serious intestinal diseases.

Phytomolecules – the intelligent alternative to antibiotics

To safeguard performance during stressful periods, poultry producers need to anticipate them and proactively provide effective gut health support. For many years, this support came in the form of antibiotic growth promoters (AGP): administered prophylactically, they were effective at keeping harmful enteric bacteria in check. However, due to grave concerns about the development of antimicrobial resistance, non-therapeutic antibiotics use has been banned in many countries. Alternatives need to focus on improving feed digestibility and strengthening gut health, attacking the root causes of why the intestinal microflora would become unbalanced in the first place.

Phytomolecules are secondary metabolites active in the defense mechanisms of plants. Studies have found that certain phytomolecules stimulate digestive enzyme activities and stabilize the gut microflora, "leading to improved feed utilization and less exposure to growth-depressing disorders associated with digestion and metabolism" (Zhai et al., 2018). With other trials showing positive effects on broilers' growth performance and feed conversion, the research indicates that phytomolecules might also specifically support chickens during challenging phases.

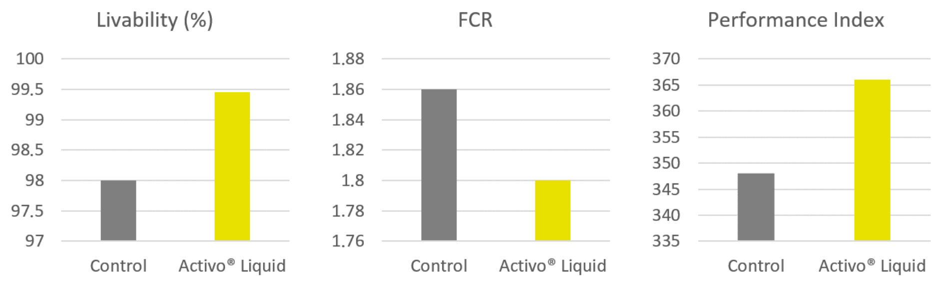
The effect of phytomolecules on broilers during a challenging phase

A study was conducted over a period of 49 days on a commercial broiler farm of an AGP-free integration operation in Japan. The farm reported gut health challenges in the second and third week of the fattening period due to vaccinations and changes to the animals' diets. The trial included 15504 Ross 308 broilers, divided into two groups. The negative control group included a total of 7242 birds, kept in another house.

All the birds were fed the standard feed of the farm. The trial group (8262 birds) received Activo Liquid, which contains a synergistic combination of phytomolecules, administered directly through the drinking water. Activo Liquid was given at an inclusion rate of 200ml per 1000L of water (3.3 US fl oz per gallon of stock solution, diluted at 1:128), from day 8 until day 25, for 8 hours a day.

The results are summarized in Figure 1:

Figure 1: Improved broiler performance for Activo Liquid group (day 49)



The Activo Liquid group clearly showed performance improvements compared to the control group. Livability augmented by 1.5%, while the feed conversion rate improved by 3.2%. This resulted in a more than 5% higher score in terms of the performance index.

Challenging times? Tackle them using phytomolecules

Poultry producers take great care to eliminate unnecessary sources of stress for their birds. Nonetheless, during their lifecycle, broiler chickens face challenging periods during which the balance of the intestinal microflora can easily become disturbed, with consequences ranging from decreased nutrient absorption to full-blown enteric disease.

The trial reviewed here showed that, after receiving Activo Liquid, broilers raised without AGPs showed encouraging performance improvements during a challenging phase of feed changes and vaccinations. Likely thanks to the activation of digestive enzymes and a stabilization of the gut flora, the broilers showed improved livability and feed conversion, thus delivering a much more robust performance during a critical phase of their lives. In times where the non-therapeutic use of antibiotics is no longer an option, phytomolecules allow poultry farmers to effectively support their animals during challenging times.

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Photo Source: Aviagen

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Phytomolecules: A tool against antibiotic-resistant E. coli

Diseases caused by E. coli entail use of antibiotics in animal production

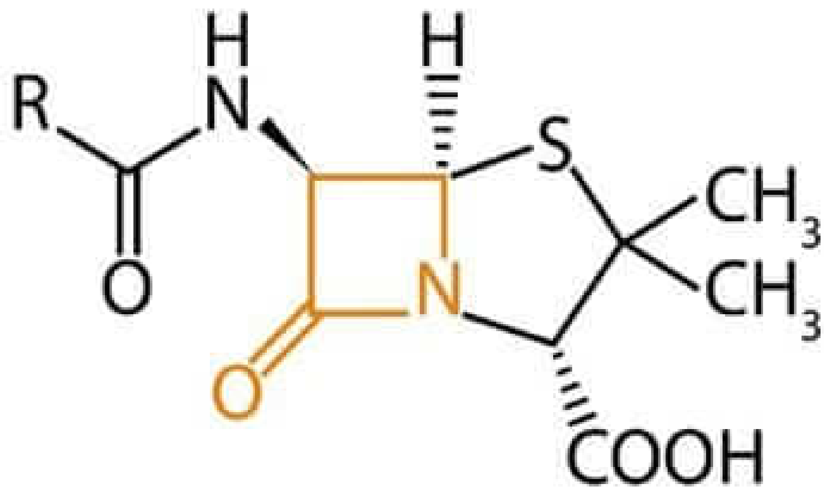
E. coli infections are a major problem in pig production. Especially young animals with an incompletely developed immune system are often unable to cope with the cavalcade of pathogens. In poultry, E. coli are responsible for oedema, but also for respiratory diseases. In young piglets, E. coli cause diarrhoea , oedema, endotoxic shock and death. In order to cure the animals, antibiotics often must be applied. Besides this curative application, antibiotics were and in many countries still are used prophylactically and as growth promoters.

The excessive use of antibiotics, however, leads to the occurrence of antimicrobial resistance (AMR): due to mutations, resistance genes are created which enable enterobacteria such as Salmonella, Klebsiella and E. coli to produce enzymes (ß-lactamases) in order to withstand ß-lactam antibiotics. In case of an antibiotic treatment, the resistant bacteria survive whereas the other bacteria die.
The major problem here is that these resistance genes can be transferred to other bacteria. Harmless bacteria can thus transfer resistance genes to dangerous pathogens, which then cannot be combatted with antibiotics anymore. In this article we explore in detail how AMR happens and how phytomolecules, which have antimicrobial properties, could be a key tool to reduce the need for antibiotics in animal production.

How ß-lactam antibiotics work

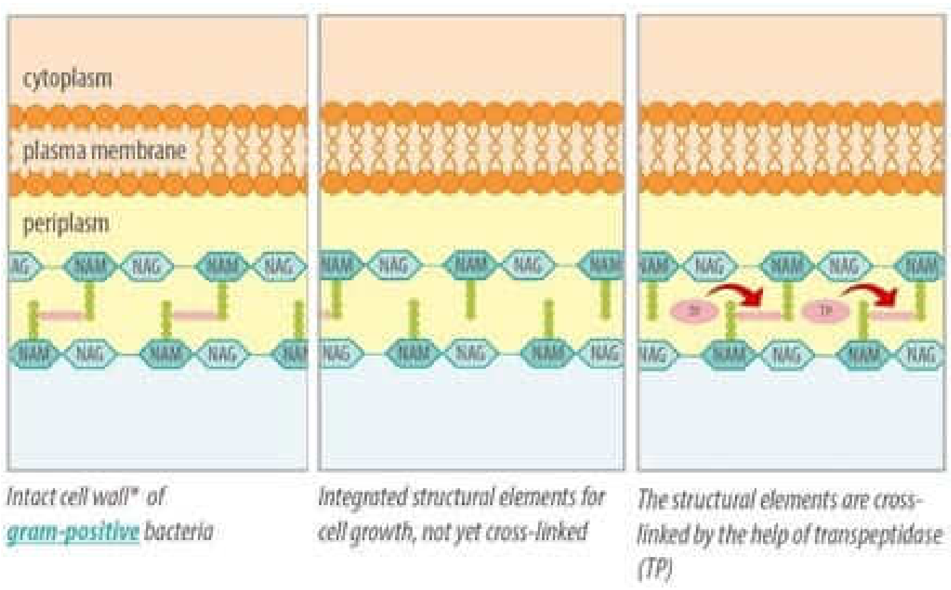
The group of ß-lactam antibiotics consists of penicillins, cephalosporins, monobactams, and carbapenems. These antibiotics are characterised by their lactam ring (Figure 1).

Figure 1: An antibiotic with a ß-lactam ring (in orange)



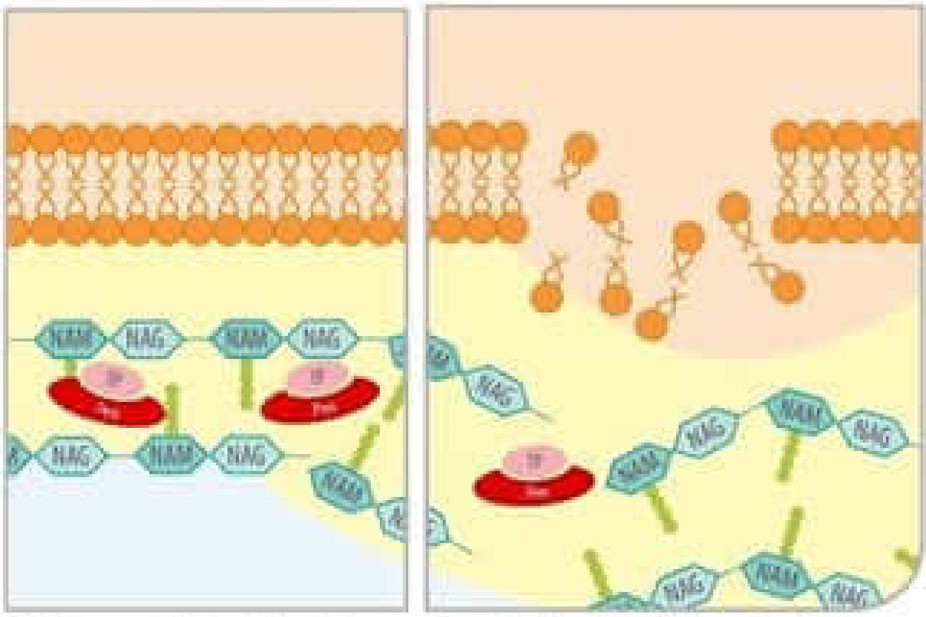
If bacteria are growing, the cell wall also has to grow. For this purpose existing conjunctions are cracked and new components are inserted. In order for the cell wall to remain a solid barrier, the new components must be interconnected by crosslinks. For the creation of these crosslinks an enzyme is essential, the transpeptidase (figure 2).

Figure 2: building up a stable cell wall with the help of transpeptidase



Due to their structure, ß-lactam-antibiotics also fit as binding partner for transpeptidase. They bind to the enzyme and block it (Kohanski et al., 2010). The crosslinks cannot be created and the stabilization of the cell wall is prevented. Disturbance of cell wall stability leads to the death of the bacterial cell, hence ß-lactam antibiotics act bactericidal.

Figure 3: blocked by ß-lactam antibiotics, transpeptidase cannot serve as enzyme for building the cell wall



If the transpeptidase is blocked e.g. by Penicillin (Pen), cross-linking is not possible

The unstable cell wall does not withstand the pressure any more, the bacteria cell gets destroyed

The challenge: E. coli producing ß-lactamases

Resistant bacteria, which are able to produce ß-lactamases – enzymes that destroy the ß-lactam ring – prevent their own destruction. Divers point mutations within the ß-lactamase genes lead to the occurrence of “extended-spectrum-beta-lactamases“ (ESBL). ESBL are able to inactivate most of the ß-Lactam-antibiotics.

Another mutation leads to so-called AmpC (aminopenicillin and cephalosporin) ß-lactamases. They enable the *E. coli* to express a resistance against penicillins, cephalosporins of the second and third generation as well as against cephamycins.

Phytomolecules – an alternative?

One approach to reduce the use of antibiotics is the utilization of phytomolecules. These secondary metabolites are produced by plants to protect themselves from moulds, yeasts, bacteria and other harmful organisms.

The use of plants and their extracts in human and veterinary medicine is well-established for centuries. Besides digestive and antioxidant characteristics they are well known for their bacteriostatic and bactericidal effects.

Consisting of a high number of chemical compounds, they attack at diverse points and their antimicrobial effect is not caused by only one single specific mechanism. This is crucial because it is therefore very unlikely that bacteria can develop resistances to phytomolecules like they do to antibiotics.

How phytomolecules work

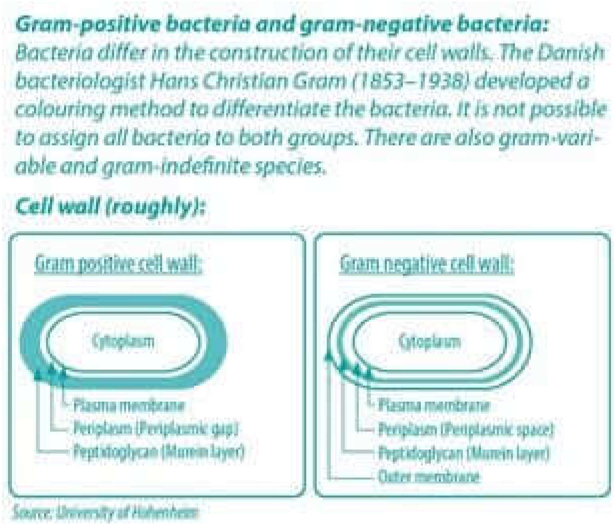
Mostly, phytomolecules act at the cell wall and the cytoplasm membrane level. Sometimes they change the whole morphology of the cell. This mode of action has been studied extensively for thymol and carvacrol, the major components of the oils of thyme and oregano.

They are able to incorporate into the bacterial membrane and to disrupt its integrity. This increases the permeability of the cell membrane for ions and other small molecules such as the energy carrier ATP

(Adenosin-tri-phosphate). It leads to the decrease of the electrochemical gradient above the cell membrane and to the loss of energy equivalents of the cell.

A special challenge: gram-negative bacteria

Gram-negative bacteria such as *E. coli* and *Salmonella* pose a special challenge. The presence of lipopolysaccharides in the outer membrane (OM) provides the gram-negative bacteria with a hydrophilic surface (Nikaido, 2003; Nazarro et al., 2013) (*see also blue infobox*).



The cell wall therefore only allows the passage of small hydrophilic solutes and is a barrier against macromolecules and hydrophobic compounds such as hydrophobic antibiotics and toxic drugs. The bypassing of the OM therefore is a prerequisite for any solute to exert bactericidal activity toward gram-negative bacteria (Helander et al., 1998).

Based on their trial results Helander et al. (1998) (1998) concluded that trans-cinnamaldehyde and partly also thymol and carvacrol gain access to the periplasm and to the deeper parts of the cell. Nikaido (1996) also concluded that OM-traversing porin proteins allow the penetration of lipophilic probes at significant rates.

Evaluating phytomolecules I – in vitro trial, Scotland

A trial conducted in Scotland evaluated the effects of Activo Liquid, a mixture of selected phytomolecules and citric acid, on ESBL-producing *E. coli* as well as on *E. coli* that generate AmpC.

Material and methods

For the trial two strains for each group were isolated from the field, a non-resistant strain of *E. coli* served as control. Suspensions of the strains with 1×10^4 CFU/ml were incubated for 6-7 h at 37°C (98.6°F) together with diverse concentrations of Activo Liquid or with cefotaxime, a cephalosporin. The cefotaxime group saved as a control for differentiating resistant and non-resistant *E. coli*.

The suspensions were put on LB agar plates and bacteria colonies were counted after further 18-22h incubation at 37°C.

Results

The antimicrobial efficacy of the blend of phytomolecules depended on the concentration at which they were used (see table 1). A bacteriostatic effect could be shown at dilutions up to 0.1 %, a bactericidal effect at higher concentrations.

Table 1: Effect of phytomolecules against resistant E. coli producing ESBL and AmpC in poultry

Poultry Microbiology Laboratory, Edinburgh, Scotland	Cefotaxime	Phytomolecules (Activo® Liquid)			
	30 µg / ml	0.1 %	0.2 %	0.4 %	0.5 %
E. coli ESBL 1 (Poultry)	-	+	++	++	++
E. coli ESBL 2 (Poultry)	-	+	++	++	++
E. coli AmpC 1 (Poultry)	-	+	++	++	++
E. coli AmpC 2 (Poultry)	-	++	++	++	++
E. coli non-resistant	+	+	++	++	++

- no effect
+ growth inhibiting (bacteriostatic)
++ bactericidal

Evaluating phytomolecules II – in vitro trial, Germany

A further trial was conducted in Germany (Vaxxinova, Münster), confirming the preceding results.

Material and methods

Four ESBL producing *E. coli* all isolated from farms and a non-resistant reference strain as control were tested concerning their sensitivity against Activo Liquid. Every bacteria strain (Conc.:1×10^4 CFU/ml) was subjected to a bacterial inhibition assay in an appropriate medium at 37°C for 6-7 hours.

Results

In this trial Activo Liquid also showed a dose-dependent efficacy, with no or just a bacteriostatic effect up to a concentration of 0.1 %, but bactericidal effects at a concentration of ≥0.2 % (table 2).

Table 2: Effect of phytomolecules against resistant ESBL producing E. coli in pig and in poultry

Vaxxinova GmbH, Münster	Phytomolecules (Activo® Liquid)			
	0.1 %	0.2 %	0.4 %	1 %
E. coli ATCC25922	+	++	++	++
E. coli ESBL 1 (Pig)	-	++	++	++
E. coli ESBL 2 (Pig)	+	++	++	++
E. coli ESBL 3 (Poultry)	+	++	++	++
E. coli ESBL 4 (Poultry)	-	++	++	++

- no effect
+ growth inhibiting (bacteriostatic)
++ bactericidal

Phytomolecules: a promising outlook

E. coli infections have devastating effects on animals, from diarrhea to edema, enterotoxic shock and even death. Antibiotic treatments have long been the only practicable answer. However, their excessive use – for instance, the metaphylactic application to thousands of animals in a flock – has led to the development of resistant strains. There is evidence that a reduction of antibiotic use reduces the occurrence of resistances (Dutil et al., 2010).

The results of the two in vitro trials in Scotland and Germany demonstrate the bactericidal effects of phytomolecules on *E. coli* that produce ESBL and AmpC. Using phytomolecules could thus reduce the use of antibiotics and therefore also the occurrence of AMR.

While it is theoretically possible for bacteria to also become resistant against phytomolecules, the probability of this happening is very low: unlike antibiotics, phytomolecules contain hundreds of chemical components with different modes of action. This makes it exceedingly difficult for bacteria to adapt and develop resistance. To tackle the problem of antibiotic-resistant *E. coli*, antimicrobial phytomolecules therefore offer a promising, sustainable and long-term solution.

By Dr. Inge Heinzl, Editor, EW Nutrition

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Phytomolecules: Boosting Poultry Performance without Antibiotics

Antimicrobial resistance (AMR) is a major threat to global public health. It is largely caused by the overuse of antibiotics in human medicine and agriculture. In intensive poultry production most antibiotics are used as antimicrobial growth promoters and/or used as prophylactic and metaphylactic treatments to healthy animals. Reducing such antibiotic interventions is crucial to lowering the incidence of AMR. However, antibiotic reduction often results in undesirable performance losses. Hence alternative solutions are needed to boost poultry performance. Phytomolecules have antimicrobial, digestive, anti-inflammatory and antioxidant properties, which could make them key to closing the performance gap.

Poultry performance depends on intestinal health

Poultry performance is to a large extent a function of intestinal health. The intestines process nutrients, electrolytes and water, produce mucin, secrete immunoglobulins and create a barrier against antigens and pathogens.

In addition, it is an important component of the body's immune defense system. The intestine has to identify pathogens and reject them, but also has to tolerate harmless and beneficial microorganisms. If the intestines do not function properly this can lead to food intolerance, dysbiosis, infections and diseases. All of these are detrimental to feed conversion and therefore also to animal performance.

Antibiotics reduce the number of microorganisms in the intestinal tract. From a performance point of view this has two benefits: first, the number of pathogens is reduced and therefore also the likelihood of diseases; second, bacteria are eliminated as competitors for the available nutrients. However, the overuse of antibiotics not only engenders AMR: antibiotics also eliminate probiotic bacteria, which negatively impacts the digestive tracts' microflora.

Products to boost poultry performance may be added to their feed or water. They range from pre- and probiotics to medium chain fatty acids and organic acids to plant extracts or phytomolecules. Especially the latter have the potential to substantially reduce the use of antibiotics in poultry farming.

Phytomolecules are promising tools for antibiotic reduction

Plants produce phytomolecules to fend off pathogens such as moulds, yeasts and bacteria. Their antimicrobial effect is achieved through a variety of complex mechanisms. Terpenoids and phenols, for example, disturb or destroy the pathogens' cell wall. Other phytomolecules inhibit their growth by influencing their genetic material. Studies on broilers show that certain phytomolecules reduce the adhesion of pathogens such as to the wall of the intestine. Carvacrol and thymol were found to be effective against different species of *Salmonella* and *Clostridium perfringens*.

There is even evidence that secondary plant compounds also possess antimicrobial characteristics against antibiotic resistant pathogens. In-vitro trials with cinnamon oil, for example, showed antimicrobial effects against methicillin resistant Staphylococcus aureus, as well as against multiresistant E. coli, Klebsiella pneumoniae and Candida albicans.

Importantly, there are no known cases to date of bacteria developing resistances to phytomolecules. Moreover, phytomolecules increase the production and activity of digestive enzymes, they suppress the metabolism of pro-inflammatory prostaglandins and they act as antioxidants. Their properties thus make them a promising alternative to the non-therapeutic use of antibiotics.

Study design and results

In order to evaluate the effect of phytomolecules on poultry performance, multiple feeding studies were conducted on broilers and laying hens. They were given a phytogenic premix (Activo, EW Nutrition GmbH) that contains standardized amounts of selected phytomolecules.

To achieve thermal stability during the feed processing and a targeted release in the birds' gastrointestinal tract, the product is microencapsulated. For each , the studies evaluated both the tolerance of the premix and the efficacy of different dosages.

Study I: Evaluation of the dose dependent efficacy and tolerance of Activo for broilers
Animals: 400 broilers; age: 1-35 days of age
Feed: Basal starter and grower diets
Treatments:
– No supplement (negative control)
– 100 mg of Activo /kg of feed
– 1.000 mg of Activo /kg of feed
– 10.000 mg of Activo /kg of feed
Parameters: weight gain, feed intake, feed conversion ratio, health status, and blood parameters

Results: The trial group given the diet supplemented with 100 mg/kg Activo showed significant improvements in body weight gain during the starter period (+4%) compared to the control group.

Additional significant improvements in feed conversion ratio (FCR) in the growing period (+4%) resulted in an overall improvement in FCR of 3%. At a 1.000 mg/kg supplementation, a significant improvement in FCR of 6% was observed over the entire feeding period. Hematological parameters were within the reference range of healthy birds when feeding up to 10,000 Activo/ kg of feed.

Study II: Evaluation of the dose depending efficacy and tolerance of Activo for laying hens

Animals: 200 hens; age: 20 to 43 weeks
Feed: basal diet for laying hens
Treatments:
– No supplement (negative control)
– 100 mg of Activo/ kg of feed
– 250 mg of Activo/ kg of feed
– 500 mg of Activo/ kg of feed
– 5.000 mg of Activo/ kg of feed
Parameters: weight gain, feed intake, feed conversion ratio, health status, and blood parameters

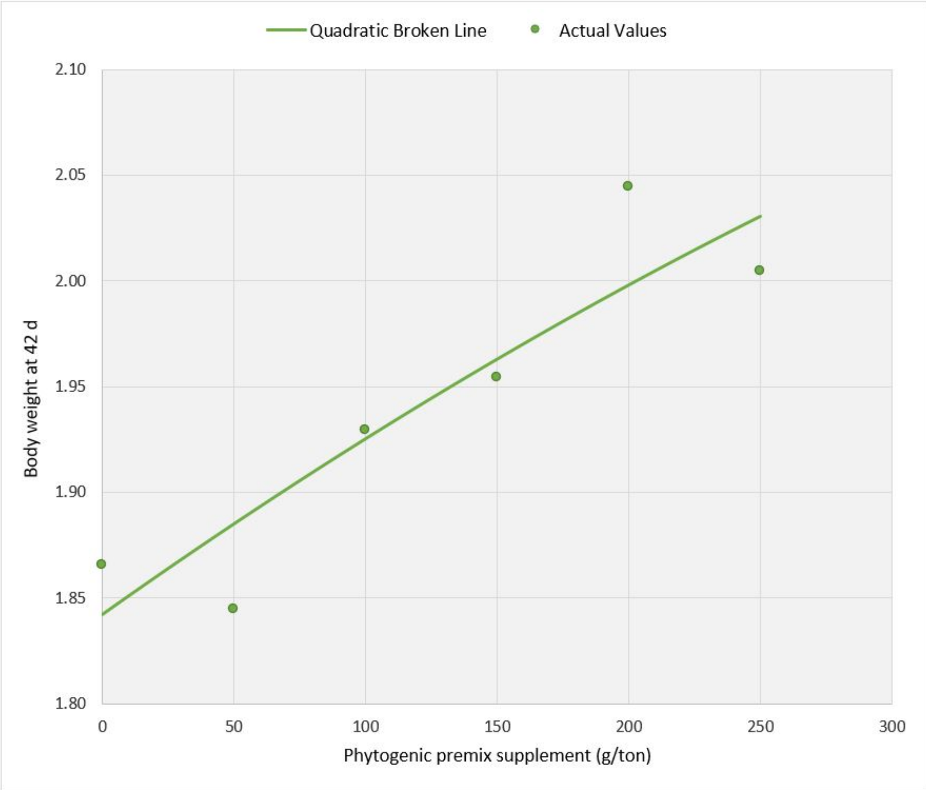
Results: Inclusion levels from 100 mg/kg of Activo onwards improved laying performance, egg mass and egg weight and reduced FCR compared to the control group. Results recorded for hematological parameters were within the reference range of healthy birds when feeding up to 5.000 mg Activo/ kg of feed.

Study III: Evaluation of the dose-dependent effects of Activo for coccidiosis vaccinated broilers

Animals: 960 broiler chickens; age: 42 days
Feed: Standard starter and finisher feed
Treatments:
– No supplement (negative control)
– 50 g of Activo /US ton of feed
– 100 g of Activo /US ton of feed
– 150 g of Activo /US ton of feed
– 200 g of Activo /US ton of feed
– 250 g of Activo /US ton of feed
– Antibiotic growth promoter (AGP)(positive control)
Parameters: weight gain, feed efficiency
Specific: In order to represent field conditions, the birds were challenged with used, homogenized litter.

Results: A clear dose response for both body weight gain and feed efficiency was observed (see Figure 1): the more phytogenic premix given, the better the birds' performance. The group with 200g of Activo /US ton of feed showed similar performance levels than the positive control group supplemented with AGP.

Figure 1: Dose-dependent effects of for coccidiosis vaccinated broilers



Study IV: Evaluation of the dose-dependent effects of Activo for laying hens

Animals: 40 hens; age: week 20 to 43
Feed: basal diet for laying hens
Treatments:
– No supplement (negative control)
– 100 mg of Activo/ kg of feed
– 250 mg of Activo/ kg of feed
– 500 mg of Activo/ kg of feed
– 5.000 mg of Activo/ kg of feed
Parameters: weight gain, feed intake, egg production, feed conversion ratio, health status
Duration: 168 days of feeding period

Results: The laying hens showed a higher laying rate when fed with a higher concentration of phytomolecules (Figure 2). Similarly improved results were observed for the feed efficiency. The more phytogenic premix added to their diet the better feed efficiency (Figure 3).

Figure 2: Dose-dependent effects of Activo on laying rate in laying hens

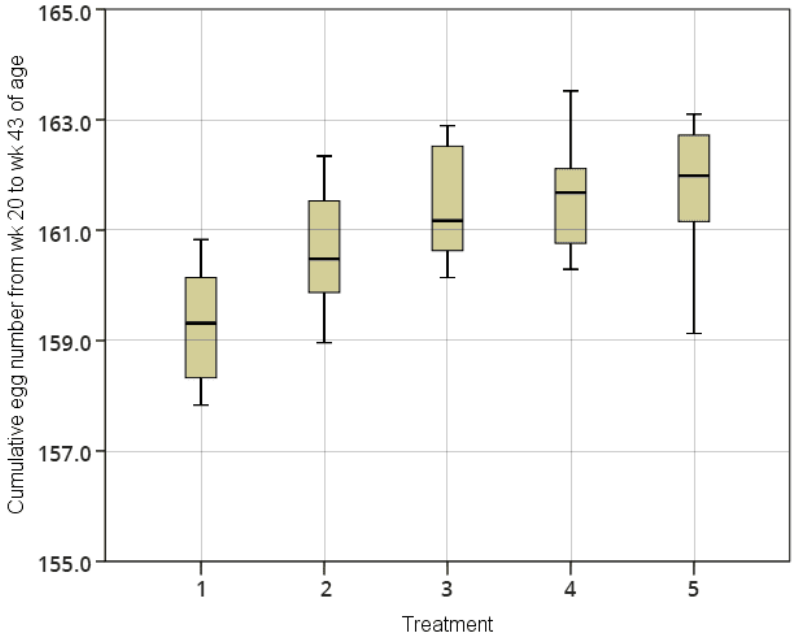
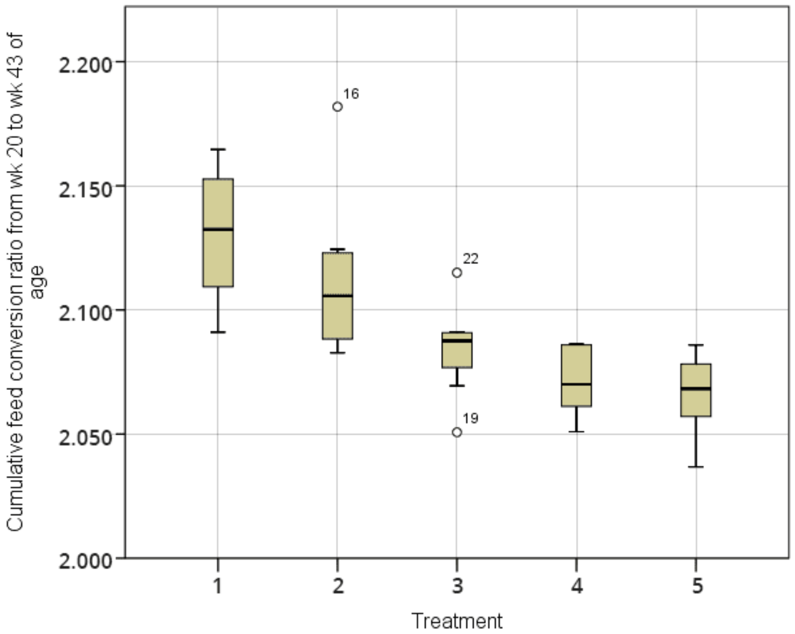


Figure 3: Dose-dependent effects of Activo on feed efficiency in laying hens



In conclusion, all four studies indicate that the inclusion of phytomolecules in broilers' and laying hens' diet improves their performance. Increasing levels of a phytogenic premix (Activo) significantly increased the production parameters for both groups. These improvements might bring performance in antibiotic-free poultry production on par with previous performance figures achieved with antimicrobial growth promoters.

The studies also showed that microencapsulated phytogenic premixes are safe when used in dose ranges recommended by the suppliers. No negative effects on animal health could be observed even at a 100 fold / 50 fold of the recommended inclusion rate in diets for broiler or laying hens, respectively. Thanks to their positive influence on intestinal health, phytomolecules thus boost poultry performance in a safe and effective way.

By Technical Team, EW Nutrition

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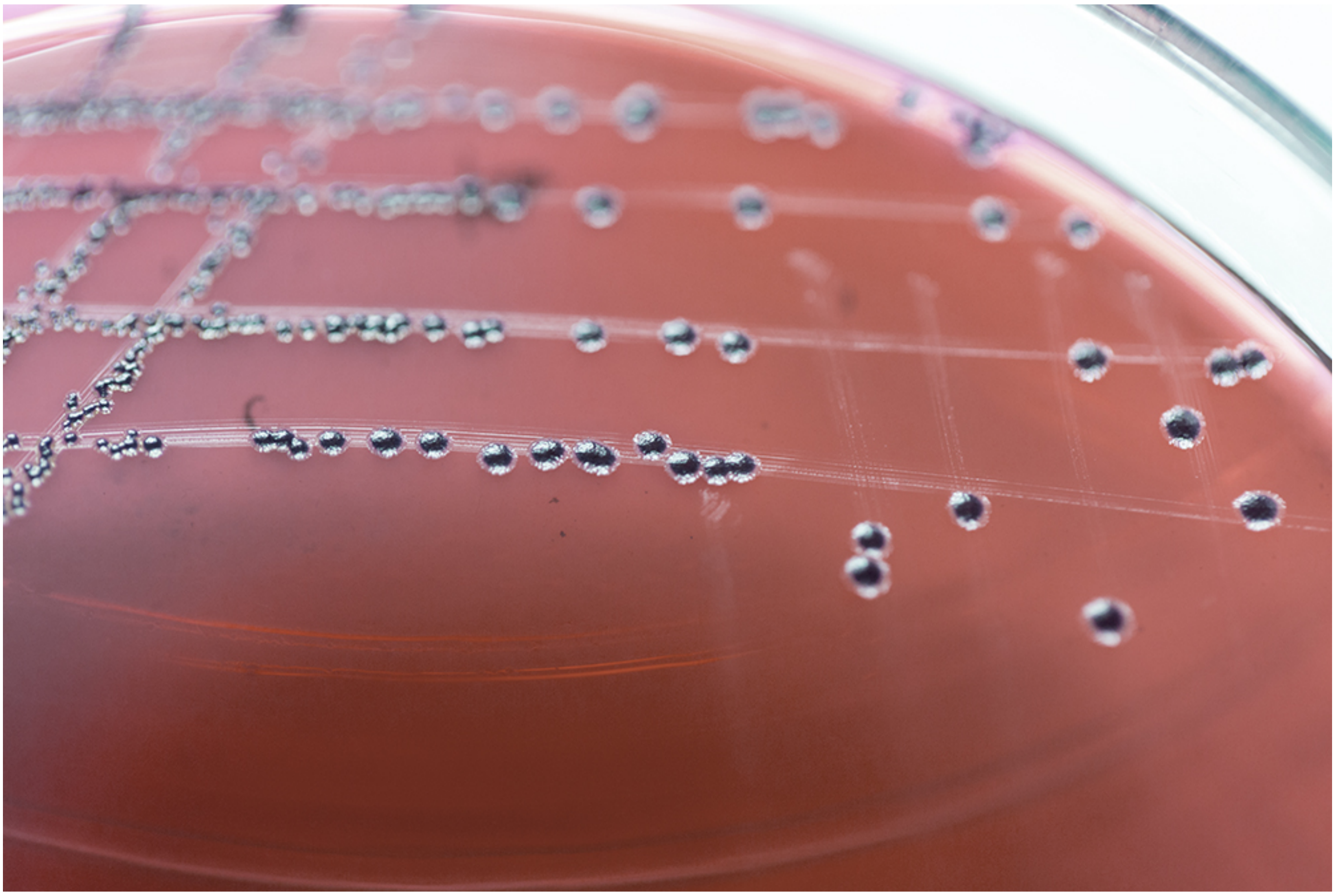
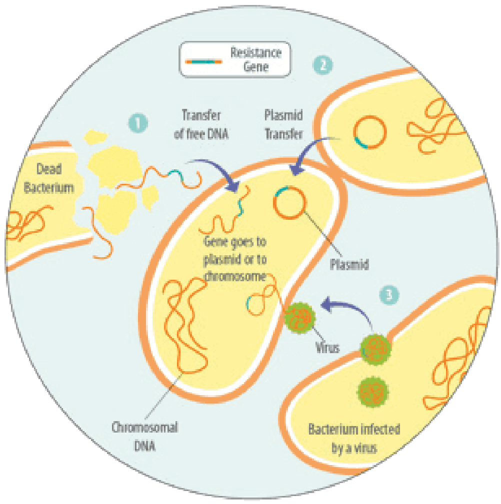
Photo source: Aviagen

Secondary Plant Compounds (SPC's) to reduce the use of antibiotics?



Initial in vitro trials give reason for hope

Antibiotic Resistance



Some bacteria, due to mutations, are less sensitive to certain antibiotics than others. This means that if certain antibiotics are used, the insensitive ones survive. Because their competitors have been eliminated, they are able to reproduce better. This resistance can be transferred to daughter cells by means of „resistance genes“. Other possibilities are the intake of free DNA and therefore these resistance genes from dead bacteria 1, through a transfer of these resistance genes by viruses 2 or from other bacteria by means of horizontal gene transfer 3 (see figure 1). Every application of antibiotics causes a selection of resistant bacteria. A short-term use or an application at a low dosage will give the bacteria a better chance to adapt, promoting the generation of resistance (Levy, 1998).

Antibiotics are promoting the development of resistance:

- Pathogenic bacteria possessing resistance genes are conserved and competitors that do not

possess these genes are killed
- Useful bacteria possessing the resistance genes are conserved and serve as a gene pool of antibiotic resistance for others
- Useful bacteria without resistance, which probably could keep the pathogens under control, are killed

Reducing the use of antibiotics
Ingredients from herbs and spices have been used for centuries in human medicine and are now also used in modern animal husbandry. Many SPC's have antimicrobial characteristics, e.g. Carvacrol and Cinnamon aldehyde. They effectively act against Salmonella, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, Entero– and *Staphylococcus*, and *Candida albicans*. Some compounds influence digestion, others act as antioxidants. Comprehensive knowledge about the single ingredients, their possible negative but also positive interaction (synergies) is essential for developing solutions. Granulated or microencapsulated products are suitable for addition to feed, liquid products would be more appropriate for an immediate application in the waterline in acute situations.

	(Activo® Liquid)		
Central Poultry Diagnostic Laboratory, Kondapur, Hyderabad (India)	10%	2%	1%
E. coli (reference strains)	++	+	+
Proteus vulgaris (reference strains)	+	+	+
Pseudomonas fluorescens	++	+	-
Salmonella pulmorum	++	++	+
Salmonella gallinarum	++	++	+
Staphylococcus aureus (reference strains)	+++	++	++

+++ 22 – 29 mm ZOI (zone of inhibition)
++ 15 – 21 mm ZOI
+ 10 – 14 mm ZOI
- < 10 mm ZOI

SPC's (Activo Liquid) against livestock pathogens in vitro
In "agar diffusion tests", the sensitivity of different strains of farm-specific pathogens was evaluated with different concentrations of Activo Liquid. The effectiveness was determined by the extent to which they prevented the development of bacterial overgrowth. The larger the bacteria-free zone, the higher the antimicrobial effect.

In this trial, Activo Liquid showed an antimicrobial effect on all bacteria tested. The degree of growth inhibition positively correlated with its concentration.

Table 1: Inhibition of field isolated standard pathogens by different concentrations of Activo Liquid

Activo Liquid against antibiotic resistant field pathogens in vitro

It cannot be excluded that resistant pathogens not only acquired effective weapons to render antibiotics harmless to them but also developed general mechanisms to rid themselves of otherwise harmful substances. In a follow-up laboratory trial, we evaluated whether the Activo Liquid composition is as effective against ESBL producing E. coli and Methicillin resistant S. aureus (MRSA) as to non-resistant members of the same species.

	SPC's (Activo® Liquid)			
Laboratory: Vaxxinova, Muenster, Germany	**0.1%**	**0.2%**	**0.4%**	**1%**
E.coli reference ATCC25922	+	++	++	++
ESBL 1 (Pig)	-	++	++	++
ESBL 2 (Pig)	+	++	++	++
ESBL 3 (Poultry)	+	++	++	++
ESBL 4 (Poultry)	-	++	++	++
S. aureus reference ATCC29213	-	+	+	++
MRSA 1 (Pig)	-	+	+	++
MRSA 2 (Pig)	-	+	+	++

- no effect
+ growth inhibiting
++ bactericide

Trial Design: Farm isolates of four ESBL producing E. coli and two MRSA strains were compared to nonresistant reference strains of the same species with respect to their sensitivity against Activo Liquid. In a Minimal Inhibitory Concentration Assay (MIC) under approved experimental conditions (Vaxxinova Diagnostic, Muenster, Germany) the antimicrobial efficacy of Activo Liquid in different concentrations was evaluated.

The efficacy of SPC's (Activo Liquid) against the tested strains could be demonstrated in a concentration-dependent manner with antimicrobial impact at higher concentrations and bacteriostatic efficacy in dilutions up to 0,1% (ESBL) and 0,2% (MRSA)(table 2).

Conclusion:

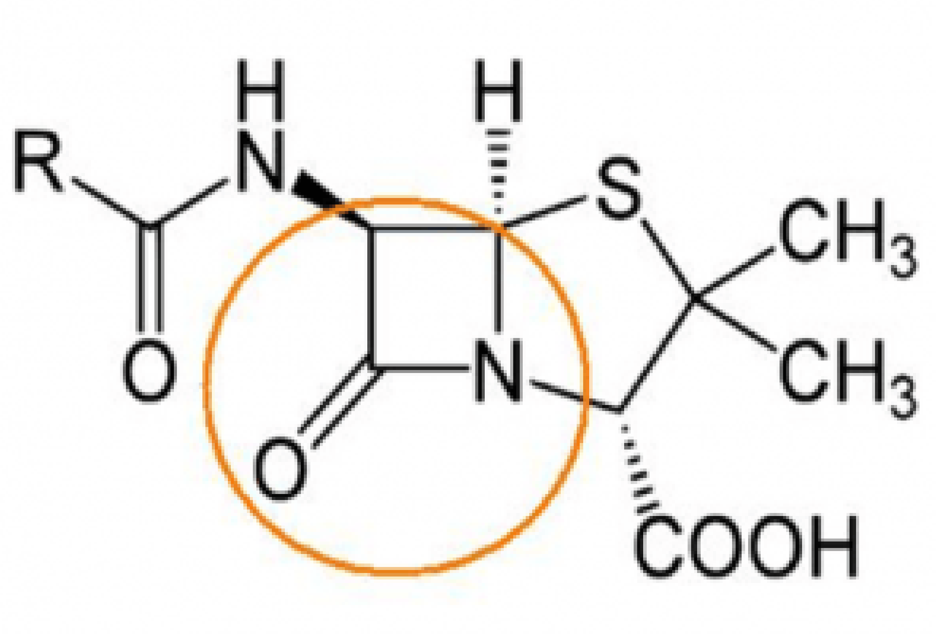
To contain the emergence and spread of newly formed resistance mechanisms it is of vital importance to reduce the use of antibiotics. SPC's are a possibility to decrease antibiotic use especially in pro- and metaphylaxis, as they show good efficacy against the common pathogens found in poultry, even against resistant ones.

I. Heinzl

Secondary plant compounds against antibiotic-resistant E. coli

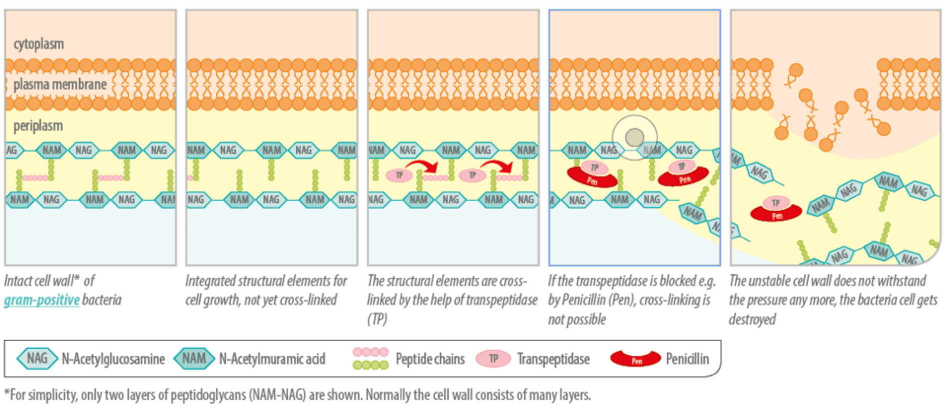
Due to incorrect therapeutic or preventive use of antibiotics in animal production as well as in human medicine, occurrence of antibiotic resistant pathogens has become a widespread problem. Enterobacteria in particular (e.g. Salmonella, Klebsiella, E. coli) possess a special mechanism of resistance. By producing special enzymes (ß-lactamases), they are able to withstand the attack of so-called ß-lactam antibiotics. The genes for this ability (resistance genes) can also be transferred to other bacteria resulting in a continuously increasing problem. Divers point mutations within the ß-lactamase genes lead to the occurrence of „Extended-Spectrum-Beta-Lactamases" (ESBL), which are able to hydrolyse most of the ß-Lactam-antibiotics. AmpC Beta-Lactamases (AmpC) are enzymes, which express a resistance against penicillins, cephalosporins of the second and third generation as well as cephamycins.

What are ß-lactam antibiotics?
The group of ß-lactam antibiotics consists of penicillins, cephalosporins, monobactams and carbapenems.
A characteristic of these antibiotics is the lactam ring (marked in orange):



Mode of action of ß-lactam antibiotic
If a bacterial cell is growing, the cell wall also has to grow. For this purpose, existing conjunctions are cracked and new components are inserted. ß-lactam-antibiotics disturb the process of cell wall construction by blocking an enzyme needed, the transpeptidase. If crosslinks necessary for the stability of the cell wall cannot be created, the bacteria cannot survive. Resistant bacteria, which are able to produce ß-lactamases, destroy the ß-lactam antibiotics and prevent their own destruction.

Secondary plant compounds
Secondary plant compounds and their components are able to prevent or slow down the growth of moulds, yeasts, viruses and bacteria. They attack at various sites, particularly the membrane and the cytoplasm. Sometimes they change the whole morphology of the cell. In the case of gram-negative bacteria, secondary plant compounds (hydrophobic) have to be mixed with an emulsifier so that they can pass the cell wall which is open only for small hydrophilic solutes. The modes of action of secondary plant compounds depend on their chemical composition. It also depends on whether single substances or blends (with possible positive or negative synergies) are used. It has been observed that extracts of spices have a lower antimicrobial efficacy than the entire spice.



The best explained mode of action is the one of thymol and carvacrol, the major components of the oils of thyme and oregano. They are able to incorporate into the bacterial membrane and to disrupt its integrity. This increases the permeability of the cell membrane for ions and other small molecules such as ATP leading to the decrease of the electrochemical gradient above the cell membrane and to the loss of energy equivalents of the cell.

Trial (Scotland)

Design

Two strains of ESBL-producing and AmpC respectively, isolated from the field, a non-resistant strain of E. coli as control. Suspensions of the strains with 1×10^4 KBE/ml were incubated for 6-7 h at 37°C together with different concentrations of Activo Liquid or with cefotaxime, a cephalosporin. The suspensions were then put on LB-Agar plates and bacteria colonies were counted after a further 18-22h incubation at 37°C. Evaluation of the effects of Activo Liquid on ESBL-producing as well as on E. coli resistant for aminopenicillin and cephalosporin (AmpC)

Results
The antimicrobial efficacy of the blend of secondary plant compounds depended on concentration with bactericidal effect at higher concentrations and bacteriostatic at dilutions up to 0,1%. It is also possible that bacteria could develop a resistance to secondary plant compounds; the probability is however relatively low, due to the fact that essential oils contain hundreds of chemical components (more than antibiotics) making it difficult for bacteria to adapt.

Phytogenics can positively influence the efficacy of antibiotics

Many veterinary antibiotics are applied via the waterline, where they are dosed in combination with other feed additives. Amongst those are mixtures of secondary plant compounds with a proven antimicrobial efficacy against veterinary pathogenic bacteria. However, little research has been done to evaluate any effect that antibiotics and phytogenics may have on each other. A possible influence of phytogenics on the efficacy of antibiotics through the combined administration would require a change in application recommendations of antibiotics and phytogenic feed additives. In the case of no interaction, no changes would be necessary. If they were to interact in a positive way, the dosages could be lowered and if they interact in a negative way, a combined application would be avoided.

Antibiotics and SPC's in co-incubation

There are different groups of antibiotics depending on the chemical structure and on the pathogen they target. Some impair the cell wall or the cytoplasmic membrane (polymyxins, ß-lactam antibiotics) and some affect protein synthesis (macrolides, Chloramphenicol, Lincospectin, tetracyclines, aminoglycosides). Others compromise DNA and RNA synthesis (fluorchinolones, ansamycines) and some disturb the metabolism of e.g. folic acid (Trimethoprim).

The intention of a trial with these different groups of antibiotics was to evaluate possible interactions they may have with a combination of secondary plant compounds. Four ESBL producing *E. coli* field isolates from poultry flocks were experimentally assessed as well as a ß-lactamase positive and a ß-lactamase negative reference strain as quality control strains for antimicrobial susceptibility testing.

Two-fold serial dilutions of antibiotics and the liquid product based on secondary plant compounds were co-incubated in a checkerboard assay. The highest concentration of the antibiotic was chosen according to CLSI standard recommendations. The control of the serial dilution of SPC's was made without antibiotics and vice versa.

Lowering the antibiotic dosage by the use of SPC's

In the experiment all field isolates proved resistant against the ß-lactam antibiotics, two field isolates and one reference strain were resistant against tetracyclines and macrolides and one field isolate and one reference strain against aminoglycocides.

The results showed that there was no negative influence of the antibiotics on the SPC's and vice versa. Moreover, for several classes of antibiotics an additive to synergistic effect was observed to such an extent that an antibiotic effect could be achieved with half or even one quarter of the former effective dosage. The dosage of the SPC-mixture could also be reduced. Based on the results of this *in vitro* experiment it can be stated that in the case of antibiotic resistance, the option exists to apply a phytogenic product with broad antimicrobial efficacy. Even more, for most combinations between antibiotics and Activo Liquid, a defined mixture of secondary plant compounds, their combined use potentiates the individual efficacy of either compound class against *E.coli* strains *in vitro*. This adds further benefits to the improvements in animal performance and health, for which a number of phytogenic feed additives have already proven effective.