# 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 <u>respiratory diseases</u>. 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.

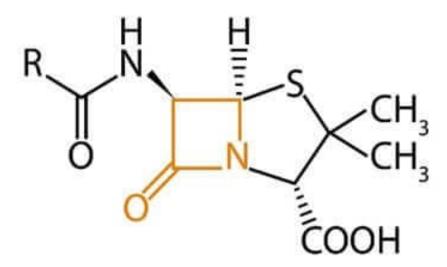
The excessive use of antibiotics, however, leads to the <u>occurrence of antimicrobial resistance</u> (AMR): due to mutations, resistance genes are created which enable enterobacteria such as Salmonella, Klebsiella and E. coli to produce enzymes (ß-lactamases) in order to withstand ß-lactam antibiotics. 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 **B-lactam** antibiotics work

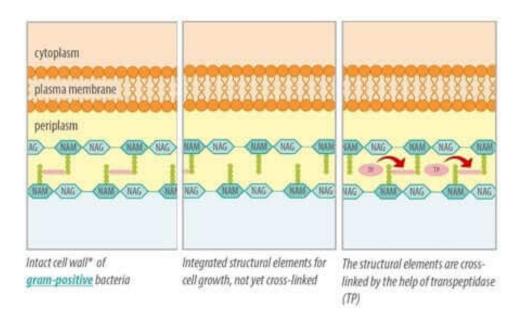
The group of ß-lactam antibiotics consists of penicillins, cephalosporins, monobactams, and carbapenems. These antibiotics are characterised by their lactam ring (Figure 1).

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



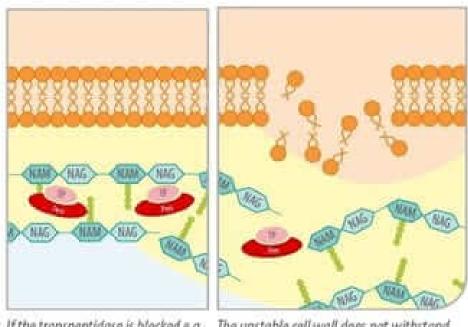
If bacteria are growing, the cell wall also has to grow. For this purpose existing conjunctions are cracked and new components are inserted. In order for the cell wall to remain a solid barrier, the new components must be interconnected by crosslinks. For the creation of these crosslinks an enzyme is 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.a. not possible

The unstable cell wall does not withstand by Penicillin (Pen), cross-linking is the pressure any more, the bacteria cell gets destroyed

## The challenge: E. coli producing ß-lactamases

Resistant bacteria, which are able to produce \( \mathbb{G}\)-lactamases - enzymes that destroy the \( \mathbb{G}\)-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-Lactamantibiotics.

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.

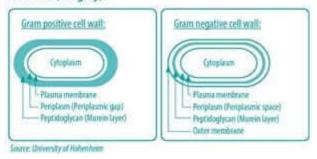
#### A special challenge: gram-negative bacteria

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).

# Gram-positive bacteria and gram-negative bacteria: Bacteria differ in the construction of their cell walls. The Danish bacteriologist Hans Christian Gram (1853–1938) developed a colouring method to differentiate the bacteria. It is not possible to assign all bacteria to both groups. There are also gram-vari-

#### Cell wall (roughly):

able and gram-indefinite species.



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 gramnegative 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  $1\times10^4$  CFU/ml were incubated for 6-7 h at  $37^{\circ}$ C ( $98.6^{\circ}$ 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, Scottland	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)		1		I

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<sup>4</sup> 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  $\geq$ 0.2 % (table 2).

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

Vaxxinova GmbH,	Phytomolecules (Activo® Liquid)				
Münster	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 <u>phytomolecules</u> 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 (<u>Activo</u>, 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)

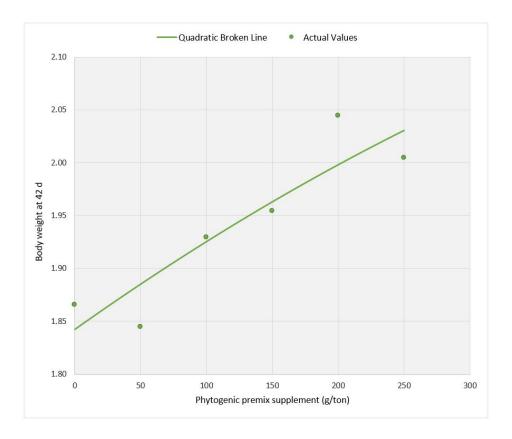
Parameters: weight gain, feed efficiency

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 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

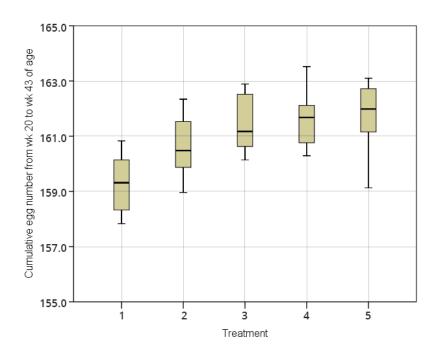
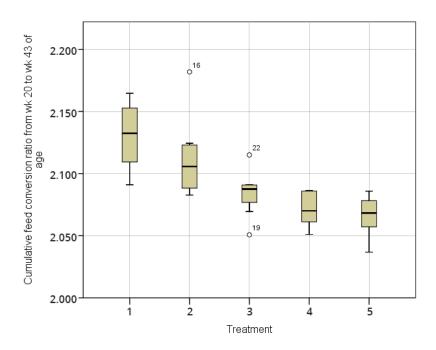


Figure 3: Dose-dependent effects of Activo on feed efficiency in laying hens



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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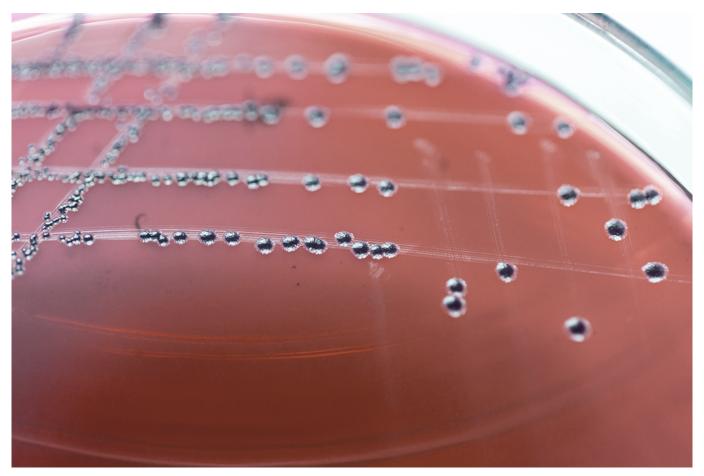
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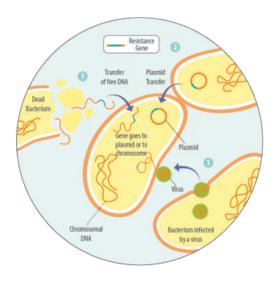
Photo source: Aviagen

# 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

	<u>Activo</u> Liquid			
Central Poultry Diagnostic Laboratory, Kondapur, Hyderabad (India)	10%	2%	1%	
E. coli (reference strains)	++	+	+	
Proteus vulgaris (reference strains)	+	+	+	
Pseudomonas <u>fluorescens</u>	++	+	-	
Salmonella <u>pulmorum</u>	++	++	+	
Salmonella gallinarum	++	++	+	
Staphylococcus aureus (reference strains)	+++	++	++	
- no effect + growth inhibiting ++ bactericidal				

Table 1: Effect of Activo Liquid against standard pathogens

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.

#### 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

	Activo Liquid			
Laboratory: Vaxxinova, Münster, Germany	0.1%	0.2%	0.4%	1%
E.coli Reference ATCC25922	+	++	++	++
ESBL I (Pig)	-	++	++	++
ESBL 2 (Pig)	±	++	++	++
ESBL 3 (Poultry)	±	++	++	++
ESBL 4 (Poultry)	-	++	++	++
S. aureus Referenz ATCC29213	-	+	++	++
MRSA I (Pig)	-	+	++	++
MRSA 2 (Pig)	-	+	+	++
- no. effect + growth inhibiting ± bacteriostatis. ++ bactericidal				

Table 2: Effect of Activo Liquid against field-isolated standard pathogens

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.

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 <u>decrease antibiotic use</u> especially in pro- and metaphylaxis, as <u>they show good efficacy against the common pathogens</u> found in poultry, even against resistant ones.

I. Heinzl

# **Necrotic enteritis in poultry**



Enteric diseases cause significant economic losses due to decreased weight gain, higher mortality, higher feed conversion, higher veterinary costs and medicine and a higher risk of contamination by poultry products in food production. The losses due to necrotic enteritis mainly occurring in broilers and fattening turkeys in intensive floor or free-range management are put at 2 billion US\$ per year.

After the ban of antibiotic growth promoters, the relevance of this formerly well controllable disease reappeared and increased.

#### Necrotic enteritis is a disease of the gut

It is caused by specific gram-positive, anaerobic bacteria – *Clostridium perfringens*, mostly Type A. Clostridia are found in litter, faeces, soil, dust and in healthy animals' guts. These spore forming bacteria are extremely resistant against environmental influences and can survive in soil, feed, and litter for several years and even reproduce.

Clostridium perfringens is a component of the normal gut flora. It occurs in a mixture of diverse strains in a concentration of up to  $10^5$  CFU / g intestinal content. In animals suffering from necrotic enteritis particularly one strain of Clostridium perfringens is found in a much more higher concentration of  $10^6$ - $10^8$  CFU / g.

Necrotic enteritis affects chickens and turkeys at the age of 2-16 weeks, proliferating at the age of 3-6 weeks. There is an acute clinical, and a subclinical form.

Birds suffering from the clinical form clearly show symptoms like a poor general state of health and diarrhoea. Mortality rates up to 50 % can occur. Subclinical necrotic enteritis cannot be diagnosed easily, as there are no clear symptoms. This form, however, stays within the flock and causes losses due to decreased growth.

#### Factors promoting an infection with necrotic enteritis should be avoided!

In general, factors have to be cited that create an intestinal environment favourable for the facultative anaerobic *Clostridium perfringens* or weaken the immune status of the host:

#### 1. Feed:

Here **NSP's** have to be mentioned. Undigested NSP's serve as substrate and some of them cause higher production of mucus also serving as substrate and providing ideal anaerobic conditions. Undigested **proteins** due to high contents in the diet also serve as substrates. **Animal protein** and **fat** are worse than vegetable variants and a homogeneous size of particles in the diet is better than an **inhomogeneous mixture**.

#### 2. Stress

Stresses such as feed change or high stocking density favour NE

#### 3. Diseases

Immunosuppressive diseases such as infective chicken anaemia, Gumboro or Marek's decrease resistance against intestinal infections and facilitate their colonisation. Some pathogens exert pressure on the gut and prepare the way for clostridia. Here Cryptosporidia and salmonella have to be mentioned.

#### **New approaches**

Secondary plant compounds show good results against the two microorganisms just mentioned. In a trial conducted with free range broilers in France, a combination of a vaccination against coccidia and a mixture of secondary plant compounds (Activo liquid) resulted in a reduced occurrence of necrotic enteritis in the trial group compared to the control. Additionally due to an improved feed conversion, the margin per animal in the trial group was 5 Cent higher than in the control  $(1,44 ext{ € vs } 1,39 ext{ €})$ .

In an *in vitro* test, Activo liquid also showed bactericidal efficacy against field isolated Salmonella pulmorum and Salmonella gallinarum at a 2 % concentration.

The trials show that combined with a good feeding and stress management, secondary plant compounds, could be a good tool to eliminate predisposing factors for necrotic enteritis and could therefore help control this economically important disease.

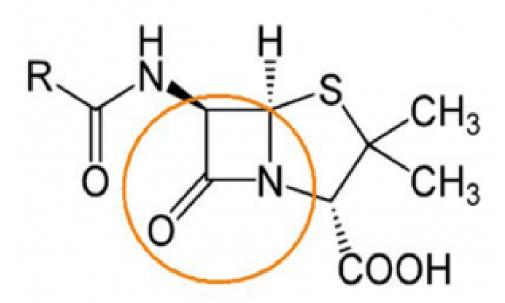
# Secondary plant compounds against antibiotic-resistant E. coli



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):

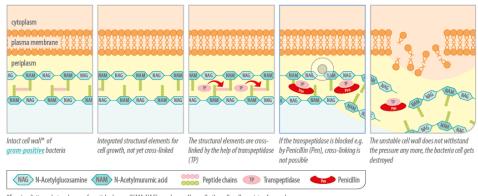


#### Mode of action of ß-lactam antibiotic

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.



 $\hbox{``For simplicity, only two layers of peptidogly cans (NAM-NAG) are shown. Normally the cell wall consists of many layers.}$ 

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  $1\times10^4$  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.