

# From sub-acute ruminal acidosis to endotoxins: Prevention for lactating cows



*by Technical Team, EW Nutrition*

Sub-acute acidosis (SARA) is linked to high levels of ruminal LPS. The LPS cause inflammation and contribute to different metabolic conditions and diseases. Various strategies and solutions can be applied to modulate the rumen microbiota and prevent this risk.



In sub-acute rumen acidosis (SARA), the quantity of free lipopolysaccharides (LPS) coming from Gram- bacteria increases considerably. These LPS cross the ruminal wall and intestine, passing into the bloodstream. The negative consequences on the health of the animal are then reflected in decreased productive and reproductive performance.

The LPS are released during the lysis of GRAM- bacteria which die due to the low pH, and these bacteria are mainly responsible for the production of propionic acid for the energy yield that is obtained. It is essential to preserve ruminal balance between Gram+ and Gram- such that there is no excess of LPS.

## Nutritional needs of lactating cows with SARA

In the first phase of lactation (from 1 week after calving to 80 – 100 days of lactation), the cow needs a high energy level to meet the large demand for milk production. This energy demand is often not fully satisfied and feed intake falls short. This deficit leads to the need to provide as much energy as possible per feed ration.

Imagine a 650 kg live weight cow, producing about 35 kg of milk per day with a fat percentage of 3.7 and a protein percentage of 3.2. To achieve this production level and fulfill its maintenance requirements, this animal needs a feed intake of 22 kg of dry matter (DM) per day, with an energy level of 21 UFL equal to 36,000 Kcal/day of NE I (Net Energy Lactation)).

To obtain an energy supply of this type, it is necessary to provide rations with a high content of cereals rich in nonstructured carbohydrates (NSC). This will allow the animals to obtain the maximum efficacy in getting the NE I from the metabolizable energy (ME) expressed as kl\*.

\* kl expresses the effectiveness in passing from EM to EN I net of the heat dissipated by the animal, therefore  $kl = ENI/EM$  (Van Es 1978).

Compared to a diet rich in NDF (Neutral Detergent Fiber), this type of diet promotes and stimulates certain strains of bacteria to the detriment of others, shifting the balance towards a greater population of bacteria that produce propionic acid instead those which produce acetic acid. This change also determines a greater share of Gram- compared to Gram+.

# What is rumen acidosis?

Rumen acidosis is that “pathology” whereby the volume of SCFA (Short Chain Fatty Acids) produced by the rumen bacteria is greater than the ability of the rumen itself to absorb and neutralize them. Rumen acidosis is mainly caused by the amylolytic and saccharolytic bacteria (*Streptococcus bovis*; *Selenomonas ruminantium*, *Bacteroides amylophilus*, *Bacteroides rumenicola* and others) responsible for the production of lactic acid. Unlike the other most representative volatile fatty acids (acetic, butyric and propionic), lactic acid has a lower pKa: 7 (3.9 versus 4.7).

This means that for the same amount of molecules produced, lactic acid releases a number of ions  $H^+$  in the fluid ten times greater than other VFAs, with evident effects on the pH.

Ruminal acidosis can be characterized as acute or subacute. During acute ruminal acidosis, the pH in the rumen drops below 4.8 and remains low for an extended period of time. Acute acidosis leads to complete anorexia, abdominal pain, diarrhea, lethargy, and eventually death. However, the prevalence of acute acidosis in dairy is very low.

## Consequences of rumen acidosis

In such situations, a series of negative consequences can be triggered in the lactating cow. Investigations (for instance, using fistulated cows) can reveal, among others, the following alteration in the rumen:

- Shift in total microbiome rumen profile (density; diversity; community structure)
- Shift in protozoa population (increase in ciliates protozoa after 3 weeks of SARA; increase in the GNB population)
- Shift in fungi population (decreasing the fungi population with high fibrolytic enzymes, which are sensitive to low pH)
- Rise in LPS rumen concentration (increasing the GNB strain and their lysis)
- Influence on the third layer of Stratified Squamous Epithelium (SSE) (desmosomes and tight junctions)
- Lower ruminal fiber degradation (reduction in the number of cellulolytic bacteria which are less resistant to acid pH)
- Reduction of the total production of fatty acids (propionic, acetic, butyric), therefore less available energy
- Lower rumen motility (also as a consequence of the smaller number of protozoa)
- **The increased acid load damages the ruminal epithelium**
- Acid accumulation increases the osmotic pressure of the rumen inducing an higher flux of water from the blood circulation into the rumen, causing swelling and rupture of rumen papilla as well as a greater hemoconcentration

The last points are extremely important, as it enables an easier passage of fluids from the blood to the pre-stomachs, greatly influencing the fermentation processes.

Furthermore, with diets low in NDF, the level of chewing and salivation is certainly lower, with a consequent lower level of salivary buffers that enter the rumen and which would maintain an appropriate pH under normal conditions.

## Rumen sub-acute and acute acidosis: a fertile ground for LPS

Studies inducing SARA in dairy cows have shown that feeding high levels of grain causes the death and cell lysis of Gram- bacteria, resulting in higher concentration of free LPS in the rumen. In a trial conducted by Ametaj et al., in 2010 (Figure 1), a lower ruminal pH and an increase in the concentration of LPS in the rumen fluid -measured as ng / ml (nanograms / milliliter)-, was the result of increasing of NSC present in the diet (% of grains).

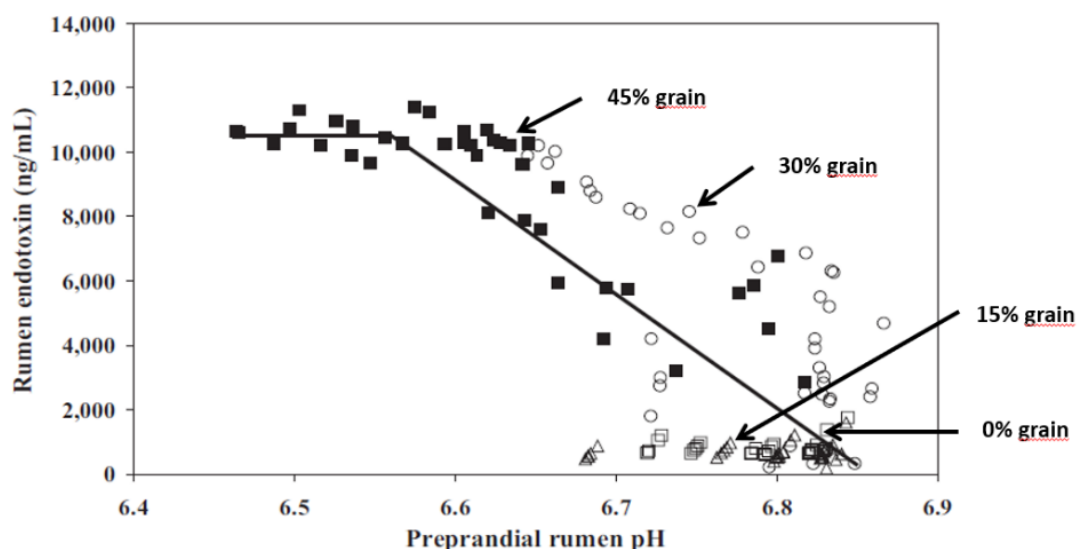


Figure 1. The increase in the level of endotoxins in the rumen is directly correlated with an increase in ration concentrates

In the rumen, the presence of Gram- is very significant, however the dietary changes towards high energy concentrates, reduce the substrates necessary for them to thrive, leading to their lysis and favoring gram-positive bacteria (Gram+). Gram+ also produce bacteriocins against a wide variety of bacteria, including many Gram-. Figure 2 shows the influence of ruminal pH in the population of different bacteria, many of which are crucial for the production of SCFA and therefore of energy.

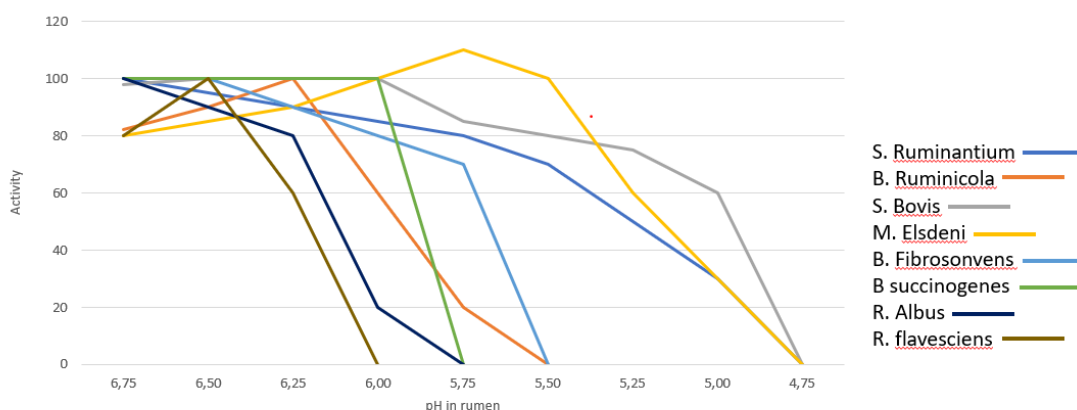


Figure 2. Activity of main bacteria in the rumen in function of pH (Daniele Cevolani Edizioni Agricole di New Business Media srl 2020)

It is therefore necessary to pay close attention to the energy level of the ration as an energy input (generally around 1500 - 1700 Kcal/kg of DM intake). At the same time, we need to ensure that the animal does receive and ingest that daily amount of DM. If ingestion is negatively influenced by acidosis (clinical or sub-clinical), this can lead to endotoxemia, with harmful consequences for the animal's health and production performance.

We can therefore note that the level of LPS (endotoxins) present in the rumen is directly correlated with the pH of the rumen itself and with a symptomatologic picture dating back to SARA. This occurs when the mortality and lysis of Gram- bacteria (GNB) is high and through the consequent imbalance created with diets containing excess fermentable starches, compared to diets with higher fiber content.

In fact, it was shown that the transition from a concentrated fodder ratio of 60:40 to a more stringent ratio of 40:60 caused the level of free LPS in the rumen to go from 410 to 4.310 EU / ml.

# Endotoxemia: Pathological consequences in dairy cows

Once the LPS enter the bloodstream, they are transported to the liver (or other organs) for the detoxification. However, sometimes this is not enough to neutralize all the endotoxins present in blood. The remaining excess can cause issues such as the modification of the body's homeostasis or cause that cascade of inflammatory cytokines responsible for the most common pathologies typical in cows in the first phase of lactation. The most common symptoms are the increase of somatic cells in milk or claws inflammation.

Pro-inflammatory cytokines as TNF, IL6 and IL8 induced by LPS-related inflammation are able to stimulate the production of ACTH (adrenocorticotrophic hormone).

ACTH, together with cortisol and the interleukins, inhibit the production of GnRH and LH, with serious effects on milk production. The productivity and the fertility of the animal are thus compromised.

Moreover, prostaglandins are as well stimulated by LPS, and are linked with fever, anorexia and ruminal stasis. This not only limits the amount of energy available for production and maintenance functions, but also induces a higher susceptibility to disease and adds-up to the emergence of other metabolic conditions, such as laminitis and mastitis.

## Preventing rumen acidosis

The solution to these massive risks is a prudent and proactive approach by the nutritionist towards all situations that can cause a rapid increase of Gram- in the rumen. It is therefore necessary to avoid cases of clinical and sub-clinical acidosis (SARA) in order to avoid the issues listed above. This would also help avoid stressful conditions for the animal that would lead to decreased performance and health.

To maintain balance and a healthy status of the animal, the use of additives such as phytomolecules and binders is suggested in the first phase of lactation, starting from 15 days before giving birth.

Activo Premium (a mix of phytogetic substances) has given excellent results in decreasing the acetic/propionic acid ratio, while safeguarding the population of Gram+ bacteria. This is in contrast to treatments with ionophores, which, as is well known, interfere with the Gram+ population.

## Case study. Acetic acid:propionic acid ratio with Activo Premium

In a study conducted at the the University of Lavras and the Agr. Res. Comp. of Minas Gerais (both Brazil), 30 Holstein cows were allocated to two groups considering parity and milk production. One group was fed the standard feed (control), the other group received standard feed containing 150mg of Activo Premium/kg of dietary dry mass (DM). The following parameters were measured or calculated: intake of DM and milk production, milk ingredients such as fat, protein, lactose every week, body weight and body condition score every two weeks, and ruminal constituents (ph and SCFAs) through oesophageal samples at day 56.

Activo Premium was able to decrease the ratio between acetic acid and propionic acid, and at the same time maintain the level of Gram+ bacteria in the rumen, thus reducing the risk of endotoxins. The same trial carried out at the University of Lavras demonstrated how the performance of the animals was superior in the group fed with Activo Premium compared to the control group (see below).

Effect of Activo Premium on ruminal constituents

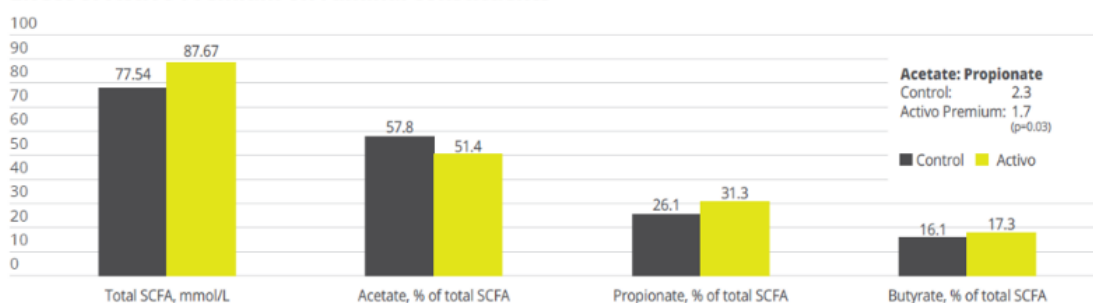


Figure 3. Effect of Activo Premium on ruminal constituents

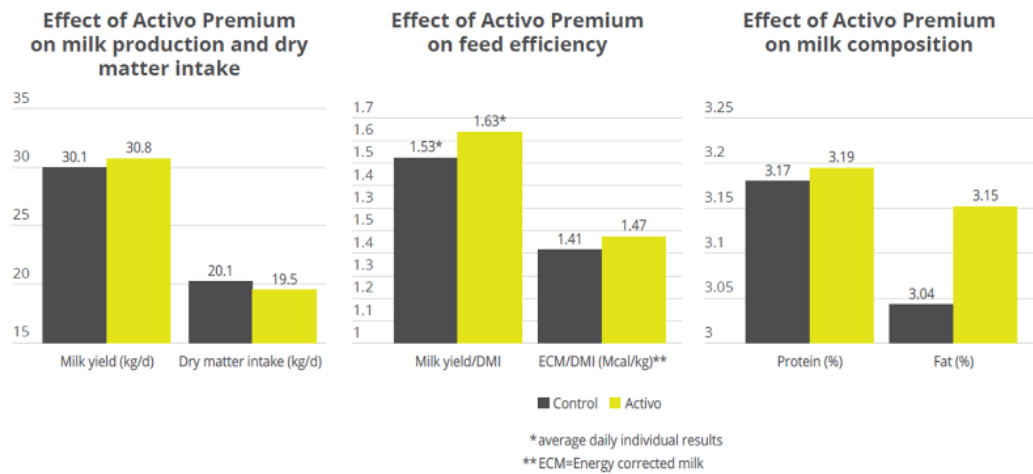


Figure 4. Effect of Activo Premium on animal performance

## Solution: Preserve Gram+ bacteria levels while decreasing free LPS

We have therefore seen how important it is to decrease the acetic:propionic ratio in the rumen to obtain a greater share of available energy. However, the level of endotoxins in the rumen must remain low in order to avoid those problems of endotoxemia linked to very specific pathologies typical of “*super productive cows*”. These pathologies (always linked to inflammatory manifestations) can be prevented by decreasing the level of free LPS in the rumen with a product that can irreversibly bind the LPS and thus make them inactive.

In a trial with porcine intestinal cells (IPEC-J2) challenged by *E. coli* LPS, a decrease in the intensity of inflammation was observed when Mastersorb Gold was added. This decrease could be shown through a lower amount of phosphorylated NF-κB in an immunofluorescence trial, as well as through the reduced production of Interleukin (IL)-8 in the cells measured by ELISA.

The fact that pig intestine tissue was used does not affect the adsorption concept. In this case, these intestinal cells are only a vehicle to demonstrate that in an aqueous solution containing 50 ng of LPS / ml and in the same solution with the addition of Mastersorb Gold, the level of LPS actually active is decreased, as a part of the LPS was tied up by Mastersorb. The solution with a lower level of LPS gave minor “inflammatory” reactions to intestinal cells, and this can be statistically reported in dairy cows.

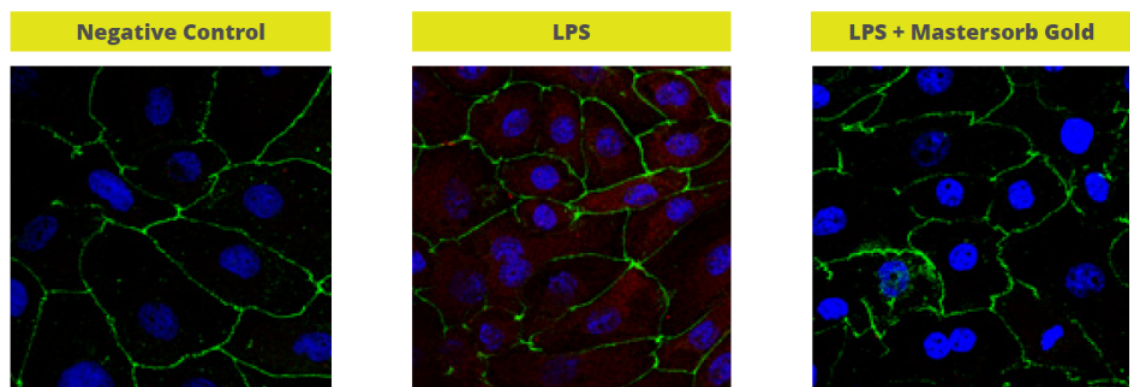


Figure 5. Immunofluorescence in IPEC-J2: Challenge with LPS without (in the middle) and with Mastersorb Gold (right)

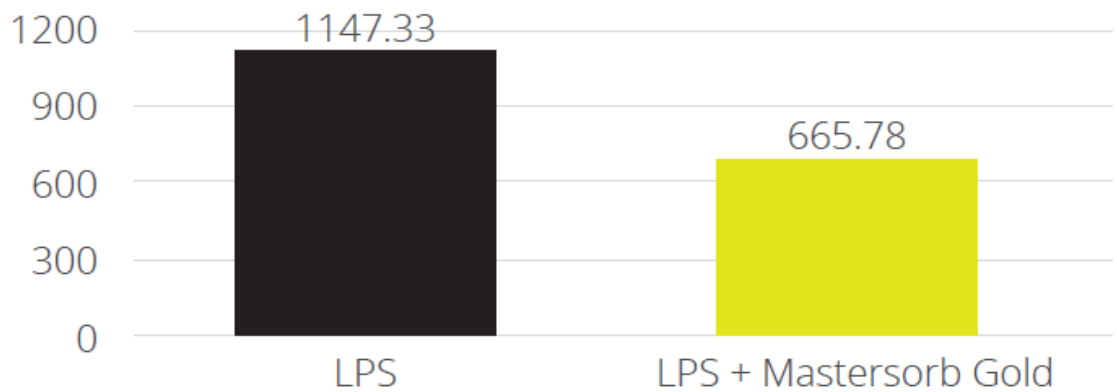


Figure 6. IL-8 AP secretion after incubation with LPS 0111:B4 for 24h without and with Mastersorb Gold

## Conclusions

To demonstrate how the decrease in the level of LPS in the rumen is directly correlated with inflammatory states in general, a trial with a total of 60 dairy cows shows that the inclusion of 25g of Mastersorb Premium/animal/day increases milk yield and improves milk quality by decreasing somatic cell count. Adsorbing substances contained in Mastersorb Premium tie up the LPS produced in the rumen in different cow lactation phases.

Normally, the rise in the level of somatic cells in milk depends on etiological agents such as *Streptococcus spp*, *Staphylococcus spp*, *mycoplasma* and more. LPS stress is not the sole agent responsible for raising somatic cell counts, but also other factors among which:

- Lactation stage and age of the animal
- Season of the year (in summer the problem is increased)
- Milking plant (proper maintenance)
- General management and nutrition

However, by reducing the level of LPS, Mastersorb provides an important aid to decrease somatic cell count.

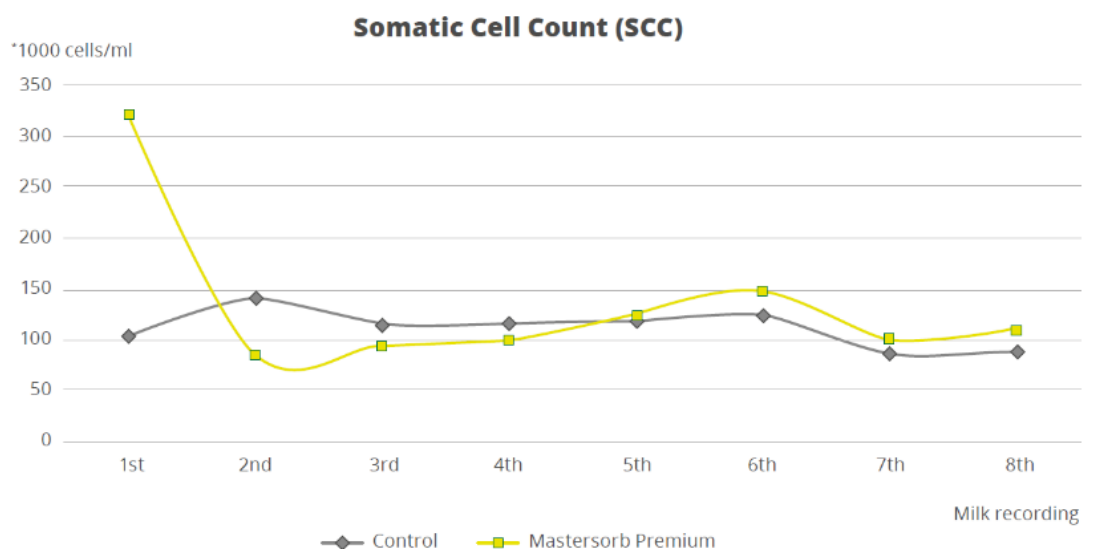


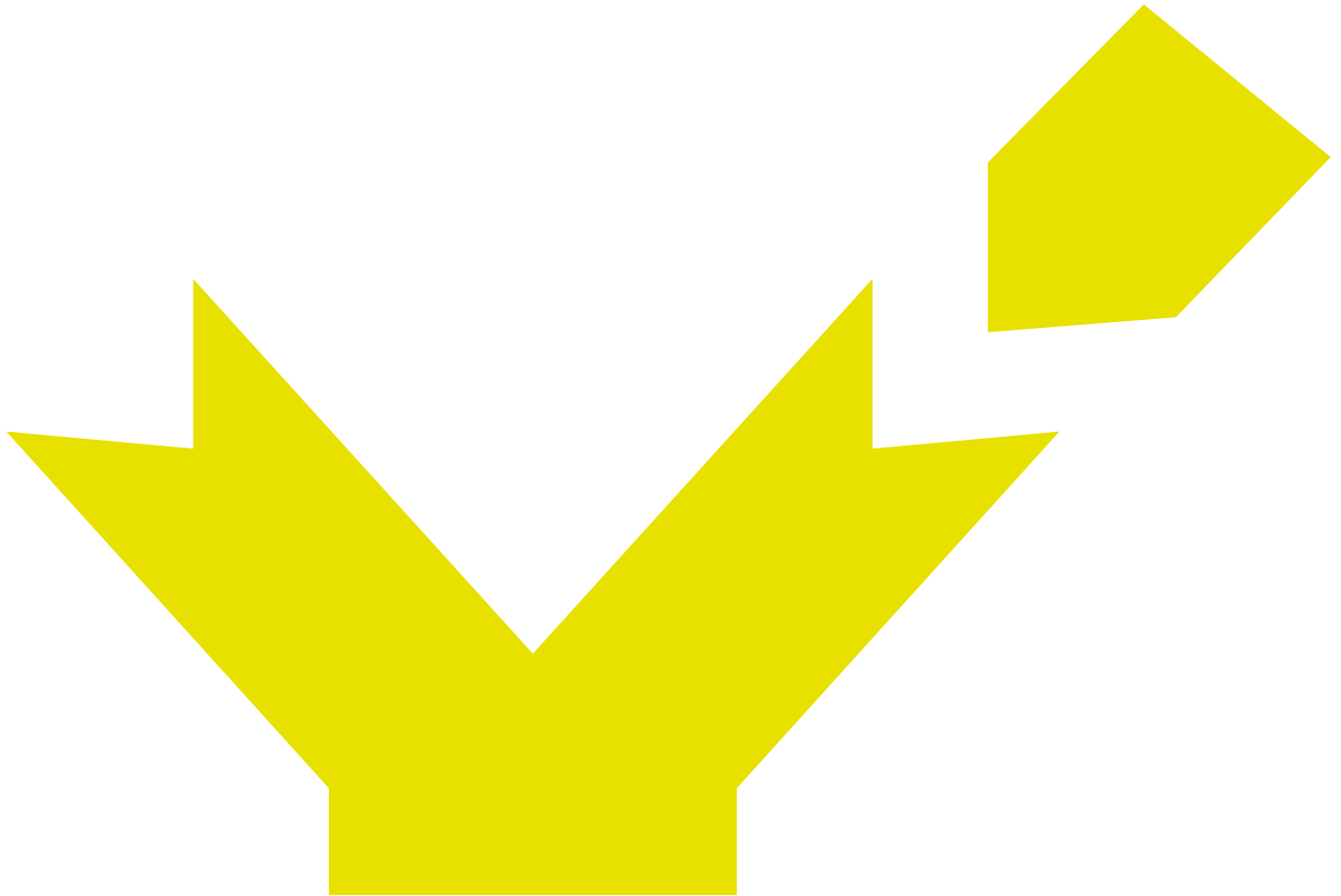
Figure 7. Effect of Mastersorb Premium on somatic cell count

# Prevent escalation with rumen balance

In the end, ruminant producers are, like all livestock operations, interested in producing healthy animals that can easily cope with various stressors. Ensuring a proper diet, adjusted to the energy requirements of various production stages, is a first step. Providing the animal with the ingredients that modulate the microbiota and reduce the negative impact of stress in the rumen is the next essential step in efficient production.

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## EW Nutrition launches new xylanase enzyme in Malaysia



**Singapore - December 28, 2020** - In its continued efforts to reduce antibiotic use in the region while also improving farm profitability, EW Nutrition launched Axxess® XY in Malaysia on December 17, 2020.



Axxess® XY is a next-generation intrinsically thermostable xylanase, with unparalleled stability under high temperatures and especially longer conditioning time. With its ability to break down both the soluble and insoluble fiber fraction from feed ingredients, the enzyme offers high flexibility in feed formulation. The newly launched product thus gives feed producers peace of mind when working with various feed processing conditions, as well as significant feed cost savings.

The launch took place in the context of a customer online event titled “Revolutions in Poultry Nutrition: The Future of Enzymes” for customers in Malaysia. During the information-laden event, Dr. Howard Simmins, an accomplished global communicator and independent key opinion leader, highlighted the need for improved functionality from xylanases. With his vast experience on feed additive development for animal nutrition and health, including cutting edge biotech research into enzymes, probiotics, prebiotics and novel additives, Dr. Simmins also discussed ways to formulate feed accurately to optimize xylanase value, and optimize nutrition with enzyme products.

During the event, other presentations were delivered by Daniel Tepe, Managing Director, Dr. Andreas Michels, Head of Biotechnology, and Dr. Ajay Awati, Global Category Manager Gut Health and Nutrition, EW Nutrition. The webinar was hosted by Jurek Grapentin, Regional Director, EW Nutrition South East Asia/Pacific.

The webinar was rounded off with an interactive Question and Answer session, where the audience had the opportunity to have their questions answered live by the panel of speakers.

“This revolutionary enzyme is a testament of our dedication to provide a holistic suite of animal nutrition solutions to our valued customers in this region. [Axxess® XY](#) will provide a competitive edge with regard to feed formulation, which translates to cost savings to our customer. With the expertise of our R&D researchers, we are constantly striving to bring more innovative solutions to meet the challenging needs of this industry,” said **Jurek Grapentin**.

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## Want antibiotic-free broilers?

## Raise low-AB breeders



Strong demand by consumers; restaurant chains and wholesalers for antibiotic-free (ABF) meat; the threat of [antimicrobial resistance](#); and stringent regulations on the use of antibiotics – there are many good reasons for poultry producers to strive for antibiotic-free production systems. Crucially, to successfully produce poultry meat without antibiotics requires a paradigm shift that starts right at the parent stock level, with the antibiotic-free production of hatching eggs.

## Broiler breeders' gut health is linked to progeny's performance

Broiler breeders' performance is measured in terms of how many saleable day old chicks (DOCs) per hen they produce. However, within a sustainable ABF production system (also known as No Antibiotics Ever or NAE), this parameter is not seen in isolation. Breeder hens' nutritional and health status not only affect the number of DOCs they can produce, but also the transfer of nutrients, antibodies, microbiota and even contaminants, e.g. mycotoxins, to the egg – and therefore, their progeny's long-term health and performance.

This starts with egg formation, which requires several metabolic processes in the hen to function perfectly. If the hen's intestinal integrity is compromised, for example due to mycotoxins, she will absorb fewer nutrients, which in turn affects egg formation. [Mycotoxicosis has particularly insidious effects for egg formation](#) as it can damage the liver whose biosynthetic activities strongly impact on the egg's internal (yolk) and external (eggshell) quality.

Chick embryos depend on the [maternal antibodies and nutrients deposited in the yolk](#), including vitamin D3, carotenoids, and fatty acids, to develop normally. Eggshell quality, among other things, affects the

embryo's access to oxygen, which is especially important when it develops body tissues.

Hens' ability to form healthy eggs depends on their diet and health. Research indicates that, via the impact on egg formation, broiler breeders' feeding program quantifiably influences their progeny's [immune system](#) and [intestinal health](#). There is indeed a direct relationship between parent and offspring's gut health because [the chick's microbiome is in part also inherited from the hen](#). The impact on DOC quality is thus one of many dimensions to consider when calibrating one's broiler breeders feeding approach.

## The challenge of feeding an ABF broiler breeder

Just as their offspring, breeder hens are genetically predisposed for rapid growth and muscle development. From rearing right through to the laying period, poultry nutritionists need to carefully balance their diets and moderate weight gain in order for hens to reach their reproductive potential.

Different stages of a breeder's life cycle come with different objectives – for example, good flock uniformity in the rearing period versus egg size and hatchability in the laying phase – and thus different requirements in terms of calories, amino acids, vitamins, and minerals. What remains constant is that the actual nutrient intake depends on intestinal health, determining both the breeders' performance and, via the impact on egg characteristics, its progeny's performance.

The [feeding regimes adopted to avoid hens becoming overweight can have a negative effect on their gut flora](#). Without antibiotics as a tool to maintain or recover optimal gut function, even mild intestinal disorders can quickly become chronic impairments that negatively impact breeders' productivity. In ABF production systems, intestinal health therefore needs to be a central focus for the feeding strategy.

## Can phytomolecules improve broiler breeders' performance?

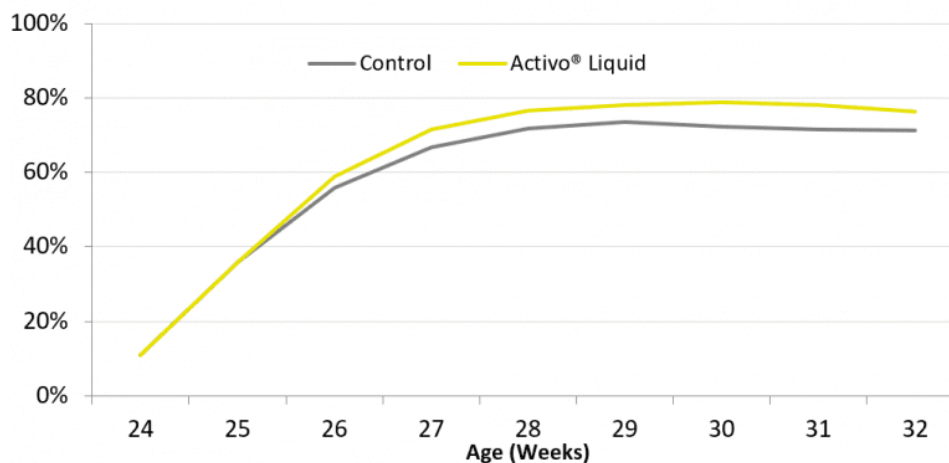
Among the plethora of feed additives, phytomolecules, or secondary plant compounds, stand out as a class of active ingredients that may help to improve gut health and thereby reduce the use of antibiotics. Synthesized by plants as a defense mechanism against pathogens, phytomolecules combine digestive, antimicrobial and antioxidant properties.

Some studies have shown that [phytomolecules-based products](#) can increase broilers' body weight gain and improve laying hens' laying rate, egg mass and egg weight. Both broilers and laying hens responded to the inclusion of phytomolecules in their diet with inclusion rate-dependent improvements in feed conversion. To evaluate if phytomolecules could similarly improve broiler breeders' performance, two trials were conducted.

### Study I: Effect of phytomolecules on laying performance during peak production

The first study was set up on a farm in Thailand. In total, 40000 Cobb broiler breeders (85% female, 15% male) were divided into two groups with 8500 hens (one house) in the control and 25500 (three houses) in the trial group. Both groups were fed standard feed. The trial group additionally received a phytomolecules-based liquid complementary feed (Activo® Liquid, EW Nutrition GmbH) via the waterline from week 24 to week 32 at a rate of 200ml/1000L during 5 days per week.

Activo® Liquid was found to have a positive influence on laying performance (Figure 1). The average laying rate increased by 7.2% during the trial period, resulting in almost 3 additional hatching eggs per hen housed. A further indication of the beneficial influence that this particular combination of phytomolecules had on gut health was a 0.2% lower mortality.



**Figure 1: Laying rate (%) of breeder hens during first 9 weeks of production**

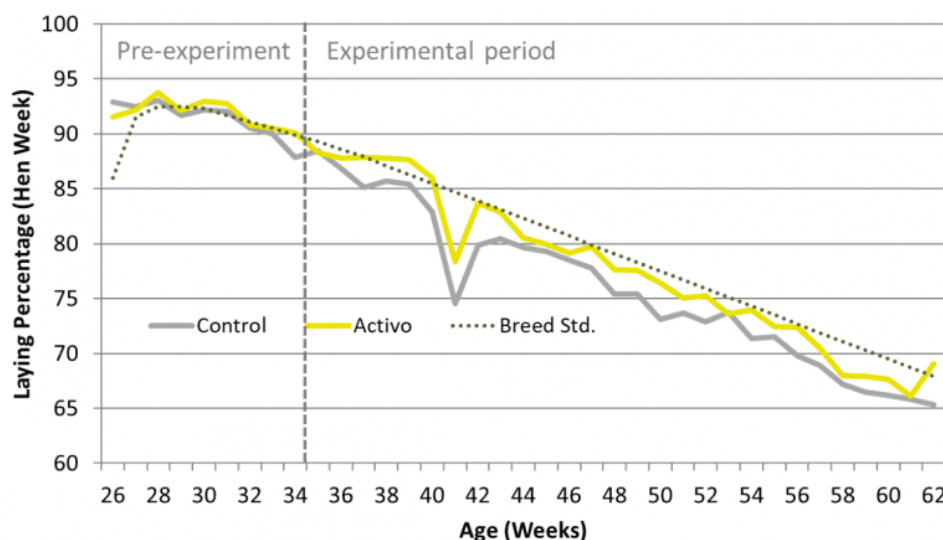
## Study II: Effect of phytomolecules on laying performance after peak production

For a second study, conducted in the Czech Republic, 800 female and 80 male Hubbard breeders (JA57 and M77, respectively) were divided into 2 groups with 5 replicate pens and 80 female and 8 male breeders per pen. The experiment started after the peak-production period, at 34 weeks of age and ended at 62 weeks of age. All animals received a standard mash diet. For one group a phytogenic premix (Activo®, EW Nutrition GmbH) was added to the diet at a rate of 100g/MT.

The results indicate that Activo® helped maintain the breeder hens' egg laying performance close to the breed's genetic potential (Figure 2). In the course of the experiment, Activo® supplemented birds produced 3.6 more eggs than control birds, while consuming a similar amount of feed. As a result, feed consumption per egg produced was lower for birds receiving phytomolecules than for the control birds (169.9 versus 173.6g/d, respectively).

As hatchability was not influenced by the dietary treatment in this study ( $P > 0.5$ ), the 3.6 extra eggs resulted in 2.9 extra day old chicks per hen produced, during the post-peak period alone.

The microencapsulated, selected phytomolecules contained in Activo® are likely to have improved gut health and feed digestibility, and thereby enhanced the animals' feed efficiency.



**Figure 2: Laying rate (%) of breeder hens week 35 till 62**

## Chicken or egg? Antibiotic-free poultry production looks at the bigger picture

To successfully produce antibiotic-free poultry meat requires a systematic re-think of each component of the production process. Broiler breeders' lay the foundation for their progeny's health and performance via the egg. Breeder hens need to be in optimal health to consistently deliver optimal eggs. Without recourse to antibiotics for maintaining or recovering intestinal functionality, an effective ABF production needs to make gut health central to its feeding approach.

The trials reviewed demonstrate that selected phytomolecules quantifiably boost breeders' laying performance, increasing the number of hatching eggs and DOCs, while reducing mortality and feed consumption per egg produced. As part of an intelligent antibiotic reduction strategy, the right phytogenic products can be potent tools to help poultry producers achieve their NAE objectives.

by S. Regragui Mazili, T. van Gerwe and M. Caballero

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# Understanding the dangers of mycotoxins for breeder hens



As the producers of hatching eggs and day-old chicks, breeding hens are the backbone of the poultry industry. Hence it is common practice to pay particular attention to this valuable asset's feed, selecting raw materials of high nutritional quality and safety. However, in any feed formulated for animals in production and reproduction, [studies show](#) that it is almost inevitable to find a certain level of mycotoxin contamination.

Mycotoxins exert toxic effects mainly on the gastrointestinal tract, liver, and kidneys and can accumulate in some tissues but also in the eggs. Mycotoxin contamination in breeder hens rations does not always lead to visible symptoms, such as when [trichothecenes cause oral lesions](#). However, it may influence productivity, egg quality, hatchery performance, as well as chick quality and immunity. Mycotoxin risk management is thus an essential part of managing breeder hens. Mycotoxins can negatively affect eggshell quality and, as a consequence, embryonic mortality.



By [Marisabel Caballero](#), Global Technical Manager Poultry at EW Nutrition.

## Type of mycotoxin and exposure time determine effect on egg production

[Mycotoxicosis in hens can cause reduced egg production](#), most likely because it causes a decrease in protein synthesis. A lower synthesis of albumin results from a degeneration of the liver tissue due to aflatoxin, ochratoxin, T2 and DON exposure. The liver then may look pale, friable and occasionally shows superficial hemorrhages.

The contamination levels at which these effects can be observed are as low as 100ppb in feed, for example, during a 21-day exposure to ochratoxin (*Figure 1*). With increasing levels of the toxin, production further decreases. A similar effect is observed when breeder hens are exposed to aflatoxins.

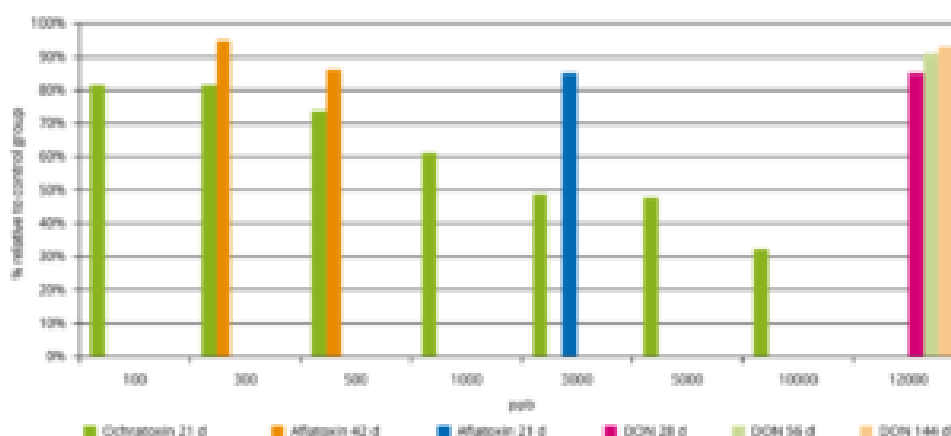


Figure 1 - Effect of mycotoxins on egg production, compared to non-contaminated control (=100 %)

Egg production, however, is not the only parameter that is affected when breeding hens are exposed to mycotoxins. Earlier on in the reproductive cycle, they already impact on embryonic mortality and hatchability. These effects are potentially more severe and may even occur without any noticeable change in the number of eggs produced.

# Mycotoxins' insidious consequences for eggshell quality and embryonic mortality

The eggshell is important to protect the progeny: thin and fragile shells can increase embryonic mortality, lower embryonic weight gain and decrease hatchability. Eggshell quality is a function of the hen's calcium and vitamin D3 metabolism. The bioavailability of calcium and of vitamin D3 depends on intestinal integrity and on the production of enzymes and transporters that aid in feed metabolism. These processes can be adversely affected by aflatoxins, DON, T2, and Fumonisin.

The gastrointestinal tract is not the only site of mycotoxin action, however. Mycotoxins such as aflatoxins and ochratoxins have nephrotoxic effects, affecting calcium metabolism and increasing its excretion via the urine, while lowering its levels in blood serum.

Moreover, mycotoxins damage the liver, which plays a central role in egg production, being responsible for vitamin D3 metabolism and the synthesis of the lipids that make up the yolk. Moreover, the synthesis of transporters for lipids, calcium, and carotenoids – important components of the egg– also takes place in the liver. When liver function is impaired, the internal and external quality of the egg declines, which, in the end, affects the production of day-old chicks.

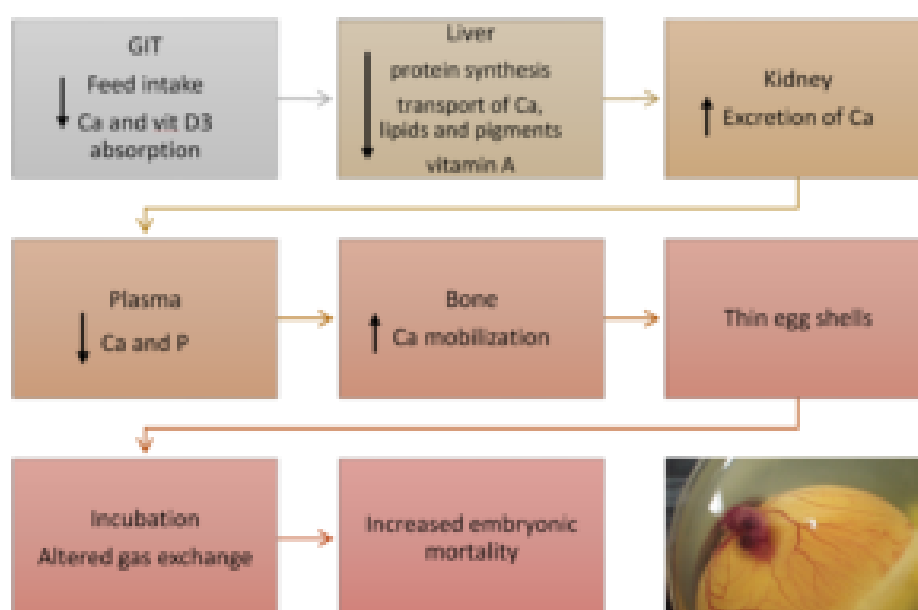


Figure 1: effects of mycotoxins on eggshell quality and embryonic mortality

Figure 2 – Effects of mycotoxins on eggshell quality and embryonic mortality

Figure 2 summarises the possible ways in which mycotoxins can negatively affect eggshell quality and, as a consequence, increase embryonic mortality. If a hen's intestinal integrity is compromised, the utilization of nutrients decreases. Liver and kidney damage leads to a diminished availability of calcium and other nutrients necessary for egg formation. The birds' calcium (and phosphorus) levels in the plasma are then lower and may lead to a [greater mobilization of calcium from the bones](#). However, this response cannot be maintained and the eggs get a thinner shell.

The thickness of the eggshell influences the egg's moisture loss and exchange with the environment during the incubation period. An eggshell of optimal quality does not allow the loss of nutrients and prevents bacterial contamination. Thinner eggshells are less able to fulfill these functions, leading to higher embryo mortality.

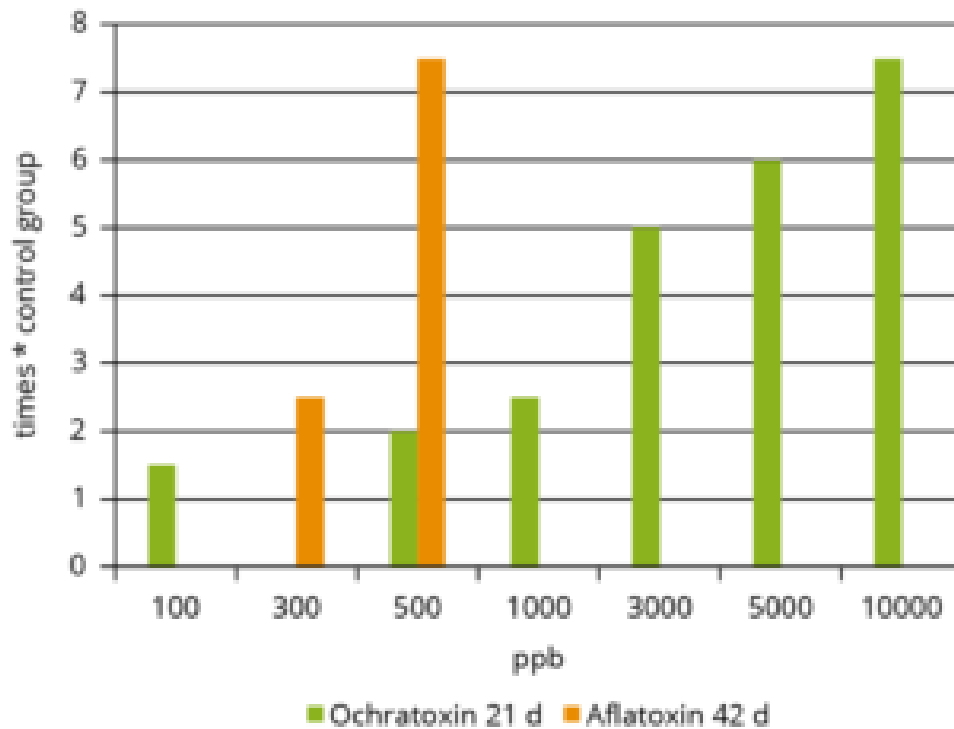


Figure 3 - Effects of mycotoxins on embryonic mortality

Figure 3 shows the effect of different mycotoxins on embryonic mortality. Incremental levels of ochratoxin or aflatoxin heighten embryonic mortality in a range from 1.5 to 7.5 times the embryonic mortality of the control group. In some cases, embryos are affected even when the hens received feed contaminated with mycotoxin levels that are within the guidelines suggested by the [EFSA](#).

For example, an exposure to 4900ppb of DON for ten weeks increases the number of embryos with abnormalities. The causes are not entirely clear, as only traces of DON can be found in the egg. However, we do know that this mycotoxin can affect the protein synthesis at the level of the hen's liver and therefore compromise the deposition of nutrients into the egg.

## Mycotoxins' effects on the progeny may cause long-term damage

Ochratoxin and aflatoxin can be transferred into the egg, where they exert toxicity on the embryos. This does not necessarily result in mortality. However, the [chicks can suffer from a compromised immune function](#) due to two reasons: lower transmission of antibodies from the hen and lower viability of the chickens' immune cells, accompanied by a lower relative weight of the bursa of Fabricio and the thymus.

When both aflatoxin and ochratoxin are present in the feed, [the effect on these parameters is synergistic](#). As a consequence of mycotoxin contamination, the animals' immune response is impaired, which makes them more susceptible to infection. The final result could be increased early chick mortality due to a higher incidence of bacterial and viral infections.

The transmission of other mycotoxins into the egg is minimal. While this means that a direct effect on the progeny is unlikely to occur, mycotoxin contamination still has a snowball effect: we have to consider the indirect effect of a lower deposition of nutrients on chick quality.

## Prevention is key: mycotoxin risk management

# for breeder hens

The best approach to manage mycotoxin risk is to implement an integrated strategy that includes good crop and grain storing practices, regular raw material sampling and mycotoxin evaluation and analysis. Management tools (such as [MasterRisk](#)) can help to evaluate mycotoxin interactions and to choose the best strategy for dealing with specific mycotoxin challenges.

The results of mycotoxin analyses can be used to take decisions regarding the inclusion levels of raw materials and in choosing [feed additives](#) that counteract mycotoxins. Products based on plant extracts, yeast cell walls, and clay minerals can help to stabilize a digestive system challenged by mycotoxins. They support the barrier function in the intestine, preventing the passage of mycotoxins into the bloodstream.

[Phytomolecules](#) are another piece of the puzzle: thanks to their antimicrobial, anti-inflammatory and antioxidant properties, they support liver function. This is particularly important for long-living animals prone to accumulating mycotoxins in their body tissues.

For a long time the “deleterious effects” of mycotoxins on breeder hens and “their repercussions on progeny health status and performance have not received from a scientific point of view as much attention” ([Calini and Sirri, 2007](#)) as they ought to have. However, now that the dangers of mycotoxins for breeder hens’ welfare, health and performance are better understood, it is clear that mycotoxin risk evaluation and management is central to successful poultry production.

\*This article first appeared in [All About Feed](#) on 31 October 2018

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# EW Nutrition Launches Top-Performing Bacterial Xylanase Enzyme

**Press Release - In December 2019, EW Nutrition has officially launched Axxess XY, a novel, intrinsically thermostable xylanase enzyme that delivers top performance to feed producers and the livestock industry. The revolutionary product was launched at a customer-centric circuit event across five locations in India.**

In its effort to improve animal gut health, control toxin risk, and reduce antibiotic use, EW Nutrition has long supported the Indian livestock industry with its holistic, science-backed solutions. The company is now entering a highly competitive market with a revolutionary solution: Axxess XY. This enzyme comes with the highest level of intrinsic thermostability and is active against both soluble and insoluble arabinoxylans. The top benefit of Axxess XY is an unparalleled flexibility in feed formulation, resulting in significant feed cost savings.

The mechanisms and derived profits of the new product were discussed during a five-city customer-centric event titled “GURU SPEAKS”, part of EW Nutrition’s “Partners in Progress” series. The key speaker was Dr. Craig Nelson Coon, Head of the Department of Poultry Sciences at the University of Arkansas, USA. Dr. Coon has over 50 years’ experience in research and teaching in poultry science.



Dr. Shirish Nigam, Managing Director of EW Nutrition South Asia, and Dr. Ajay Awati, Global Category Manager, Enzymes, highlighted Axxess XY’s unique value proposition and shared various trials conducted to prove the competitive advantage over other enzymes available in the market.

Dr. S. Mahendran, Regional Technical Manager, South Asia threw light on feed formulation optimization and explained how the addition of Axxess XY can help release additional energy from feed, which results in optimum performance and production.

The unparalleled thermostability of Axxess XY became a talking point among the audience and various integrators showed their interest in using the new enzyme in their formulations. Also, EW Nutrition’s efforts to bring everyone together on a knowledge-sharing platform was highly applauded by the attendees. Industry partners also iterated the need for more detailed sessions in the future.

The “GURU SPEAKS” series was a great opportunity to reach a wider array of breeders and broiler

integrators across India. The event was kickstarted on 16th December, 2019 at Karnal, followed by Coimbatore, Hyderabad, Pune, and Bangalore. Prof. Coon shared relevant and practical solutions to the common challenges faced by broilers and broiler breeders. He also shared his insights on nutrition and management of modern broiler breeders and highlighted that maintaining the protein balance is nowadays gaining more importance than energy requirements for better production and performance of birds. He deliberated on the pros and cons of current feeding practices and management, including biosecurity and lighting schedules for birds.

Major key stakeholders – technical consultants, university professors, farm managers, integrators, etc. – attended the series and benefitted from the information provided during the event.

“We are pleased to bring our revolutionary enzyme solution to our Indian partners, thus enriching our portfolio of products and services to the benefit of the local livestock industry,” says Michael Gerrits, Managing Director, EW Nutrition. “We are confident that Axxess XY will be a breakthrough for our customers, and we look forward to providing and servicing our comprehensive animal nutrition solutions in India, a most valuable and respected market.”

#### **About EW Nutrition**

EW Nutrition is an international animal nutrition company that offers integrators, feed producers, and self-mixing farmers comprehensive animal nutrition solutions for gut health, antibiotic reduction, young animal nutrition, toxin risk management and more.

#### **Press contact**

Ashish Sachdeva

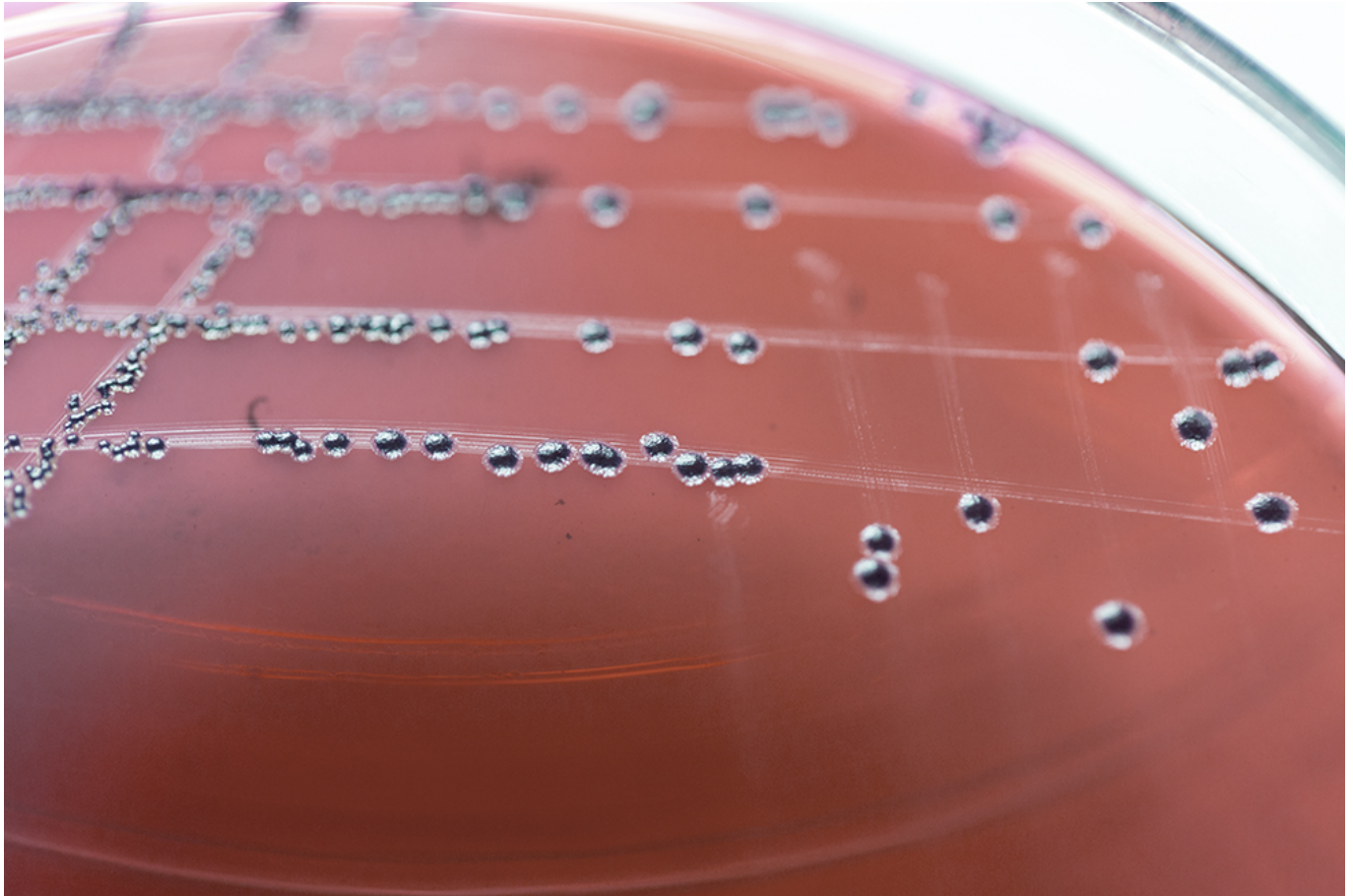
Marketing Manager, India

Email: [ashish.sachdeva@ew-nutrition.com](mailto:ashish.sachdeva@ew-nutrition.com)

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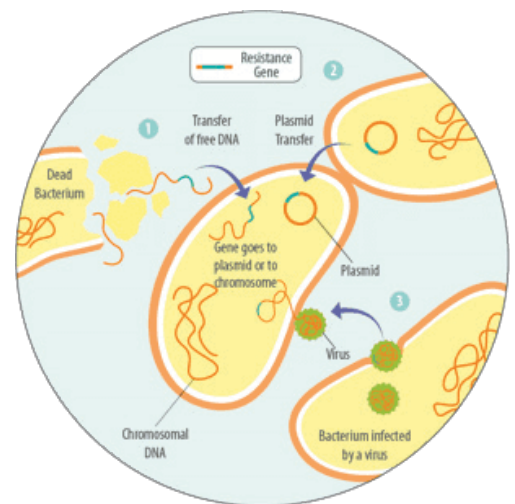
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## **Secondary Plant Compounds (SPC's) to reduce the use of antibiotics?**



**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*, Enter- 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)		
	10%	2%	1%
<i>Central Poultry Diagnostic Laboratory, Kondapur, Hyderabad (India)</i>			
<i>E. coli</i> (reference strains)	++	+	+
<i>Proteus vulgaris</i> (reference strains)	+	+	+
<i>Pseudomonas fluorescens</i>	++	+	-
<i>Salmonella pulmorum</i>	++	++	+
<i>Salmonella gallinarum</i>	++	++	+
<i>Staphylococcus aureus</i> (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.

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

# 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 € vs 1,39 €).

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.

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# **Secondary plant compounds are the new frontier in poultry nutrition**



Why should you read another story about phytochemicals? Or, is it botanicals, spices, herbs, and extracts? No matter what we call them, scientists have named them "[secondary plant compounds](#)", and if we are to follow the American tradition we can call them SPC. Then, here is the first interesting thing we can discuss about this plant-derived class of active compounds. They are "secondary" in nature, but not insignificant. They play no role in normal metabolism, but they help plants (and now animals) survive under adverse conditions. Perhaps, this is why some experts consider them as the next frontier in poultry nutrition. With [poultry](#) that are raised in less than ideal conditions, especially when we consider the movement towards antibiotic reduction (for growth promoting reasons, not complete removal of all medicines), we understand that such natural compounds can be of significant help.

As it happens, the majority of poultry specialists in Europe and increasingly in the Americas consider SPC as an almost-essential element in diets for broilers and layers (and turkeys, ducks, and all poultry for that matter) when birds are raised without antibiotics. Some go even further and use them along with antibiotics because, as we all know, antibiotics are never 100% efficient as bacteria sooner or later develop some form of resistance. Such resistance has not yet been observed with SPC. So if one is to use SPC in poultry feeds, which ones to buy? A quick glance at the market will reveal more commercial products than can possibly be imagined. Some must be better than the rest, but how can we separate the wheat from the chaff? Price alone is not always a good indicator. A high quality product must be expensive – for there is no such thing as a free lunch – but all expensive products are not always of the highest possible quality!

There are three basic criteria, which we can mention briefly here:

1. **SPC are volatile** – at least most of them. As such, unprotected products will soon evaporate if left in the open air as it happens with feed prepared in commercial farms. So, some form of protecting SPC is essential.
2. **SPC are innumerable** – so finding the right mix for the job required is important. You cannot get the same results with any kind of mix. So, in designing an SPC mix, the manufacturer must declare and have knowledge of the target to be accomplished.
3. **SPC are powerful** – meaning you cannot just keep adding as much as possible. Here finding the exact dosage for the right purpose is a difficult balancing exercise. So, the right mix and the right dosage must be combined, otherwise animals will refuse the feed (worst case scenario) or just fail to benefit from SPC inclusion.

There is so much more to learn about this exciting class of compounds that can replace the growth promoting action of antibiotics that it is worth spending time learning more about them.

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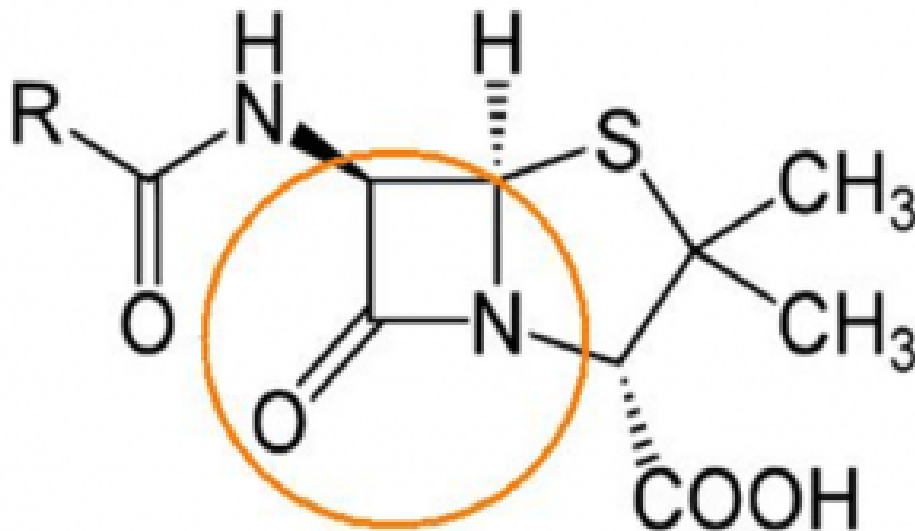
## Secondary plant compounds against antibiotic-resistant *E. coli*



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 ( $\beta$ -lactamases), they are able to withstand the attack of so-called  $\beta$ -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  $\beta$ -lactamase genes lead to the occurrence of „Extended-Spectrum-Beta-Lactamases“ (ESBL), which are able to hydrolyse most of the  $\beta$ -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 $\beta$ -lactam antibiotics?

The group of  $\beta$ -lactam antibiotics consists of penicillins, cephalosporins, monobactams and carbapenems. A characteristic of these antibiotics is the lactam ring (marked in orange):

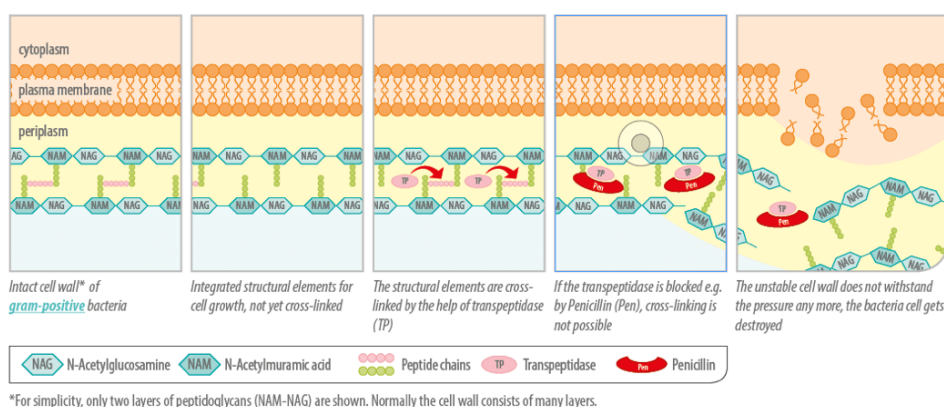


### Mode of action of $\beta$ -lactam antibiotic

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.  $\beta$ -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  $\beta$ -lactamases, destroy the  $\beta$ -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  $1 \times 10^4$  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.

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## 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 phytochemicals may have on each other. A possible influence of phytochemicals on the efficacy of antibiotics through the combined administration would require a change in application recommendations of antibiotics and phytochemical 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,  $\beta$ -lactam antibiotics) and some affect protein synthesis (macrolides, Chloramphenicol, Lincospectin, tetracyclines, aminoglycosides). Others compromise DNA and RNA synthesis (fluorquinolones, ansamycins) 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  $\beta$ -lactamase positive and a  $\beta$ -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  $\beta$ -lactam antibiotics, two field isolates and one reference strain were resistant against tetracyclines and macrolides and one field isolate and one reference strain against aminoglycosides.

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 phytochemical 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 [phytochemical feed additives have already proven effective](#).