Effective phytomolecules combine superior processing stability and strong action in the animal

*By **Dr. Inge Heinzl**, Editor, and **Dr. Ruturaj Patil**, Global Product Manager – Phytogenics, EW Nutrition*

For millennia, plants have been used for medicinal purposes in human and veterinary medicine and as spices in the kitchen. Since the ban of antibiotic growth promoters in 2006 by the European Union, they also came into focus in animal nutrition. Due to their digestive, antimicrobial, and gut health-promoting characteristics, they seemed an ideal alternative to compensate for the reduced use of antibiotics in critical periods such as brooding, feed change or gut-related stress.

To optimize the benefits of phytomolecules, it is crucial that

- the phytomolecules levels are standardized for consistent results and synergy
- they show the highest stability during stringent feed processing; being often highly volatile substances, they should not get lost at high temperatures and pressure
- the phytomolecules are preferably completely released and available in the animal to achieve the best effectiveness.

First step: Standardized phytomolecules

Essential oils and other phytogenics are sourced from plants. The composition of the plants substantially depends on genetic dissimilarity within accessions, plant origin, the site conditions, such as weather, soil, community, and harvest time, but also sample drying, storage, and extraction processes (Sadeh et al., 2019; Yang et al., 2018; Ehrlinger, 2007). For example, the oil extracted from thyme can contain between 22 and 71 % of the relevant phenol thymol (Soković et al., 2009; Shabnum and Wagay, 2011; Kowalczyk et al., 2020).

Modern technology enables the production of standardized phytomolecules with the highest degree of

purity and lowest possible batch-to-batch variation for high-quality products. It also offers increased environmental and economic sustainability due to reliable and cost-effective sourcing technology.

Using such highly standardized phytomolecules enables the production of phytogenic-based feed supplements of consistently high quality.

Second step: Selection of the most suitable phytomolecules

Phytomolecules have different primary characteristics. Some support digestion (Cho et al., 2006, Oetting, 2006; Hernandez, 2004); others act against pathogens (Sienkiewitz et al., 2013; Smith-Palmer et al., 1998; Özer et al., 2007) or are antioxidants (Wei and Shibamoto, 2007; Cuppett and Hall, 1998). To optimize gut health in animal production, one of the main promising mechanisms is reducing pathogens while promoting beneficial microbes. The decrease of pathogens in the gut not only decreases the risk of enteritis incidence but also eliminates the inconvenient competitors for feed.

In order to find out the best combination serving the intended purpose, a high number of different phytomolecules need to be evaluated concerning their structure, chemical properties, and biological activities first. Availability and costs of the substances are further factors to consider. With the selection of the most suitable phytomolecules, different mixtures are produced and tested for their effectiveness. Here, it is essential to concern synergistic or antagonistic effects.

For an effective and efficient blend of phytomolecules, many steps of selection and tests are necessary – and as a result, possibly only a few mixtures can meet the requirements.

Third step: Protecting the ingredients

Many phytomolecules are inherently highly volatile. So, only having a standardized content of phytogenics in the product can not ensure the full availability of phytomolecules when used through animal feed. Some parts of the ingredients might already get lost in the feed mill due to the stringent feed hygienization process followed by feed millers to reduce pathogenic load. The heating is a significant challenge for the highly-volatile components in a phytomolecule-based product. So, protecting these phytomolecules becomes imperative to guarantee that the phytomolecules put into the feed will reach the animal.

A delicate balancing act is required to ensure the availability and activity of phytomolecules at the right site in the gut. The phytomolecules must not get lost during feed processing but must also be released in the intestine. A carrier with capillary binding of the phytomolecules together with a protective coating can be one of the available effective solutions. It protects the ingredients during feed processing, and ensures the release in the animal.

Study shows excellent stability of Ventar D under challenging conditions

Ventar D is a latest generation phytomolecule-based solution for gut health optimization introduced by EW Nutrition, GmbH. A scientific study was conducted to compare the stability of Ventar D, in the pelleting process, with two leading phytogenics competitor feed supplements.

For this trial, feed with the different added phytogenic feed supplements had to undergo a conditioning and pelletization process. The active ingredients were analyzed before and after the pelletization process. All phytogenic feed supplements under testing were added to standard broiler feed at the producer's recommended inclusion rate. The tests took place under conditioning times of 45, 90, and 180 seconds and pelleting temperatures of 70, 80, and 90°C (158, 176, and 194°F). After cooling, triplicate samples were collected and analyzed. The respective marker substance was analyzed through gas chromatography/mass spectrometry (GC/MS) analysis to measure the recovery rate in the finished feed. The phytomolecule content of the mash feed (before pelletization) found by the laboratory was used as a baseline and set to 100% recovery. The recovery rates of the pelleted feed were evaluated relative to this baseline.

The results are presented in figure 1. Ventar D showed the highest stability of active ingredients with recovery rates of 90% at 70°C/45 sec. or 80°C/90 sec and 84% at 90°C/180 sec. The modern production technology used for Ventar D ensures that the active ingredients are well protected throughout the

pelletization process.

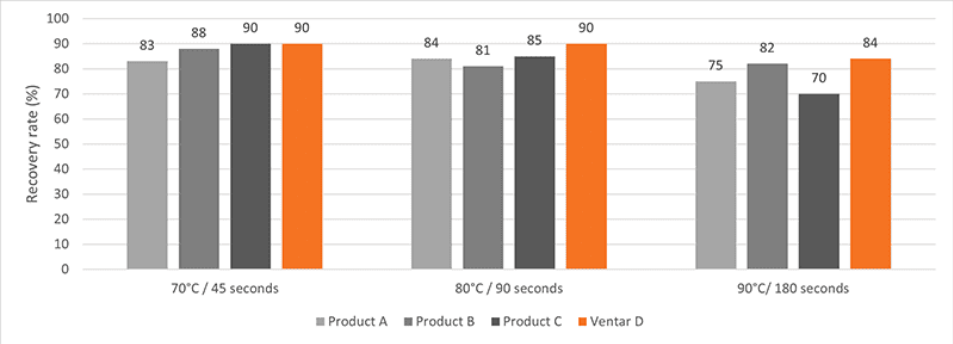

Figure 1: Phytomolecule stability under processing conditions, relative to mash baseline (100%)

Another trial was conducted in a feed mill in the US. For this trial, ten samples were collected from different batches of mash feed where Ventar D was added at 110g/t. Conditioning of the mash feed was at 87.8°C (190°F) for 6 minutes and 45 seconds. After the pelleting process, ten samples from the pelleted feed were collected from the continuous flow with a 5 min gap between the samplings to determine Ventar D's recovery.

The average recovery achieved for Ventar D was 92%.

Trials show improved growth performance

Initial trials showed Ventar D's complete release in digestion models. To examine the benefit in in-vivo conditions, Ventar D was tested in broilers at an inclusion rate of 100 g/MT.

Several in vitro studies proved the antimicrobial activity of Ventar D. One test also confirms that Ventar D could exhibit differential antimicrobial activity by having stronger activity against common enteropathogenic bacteria while sparing the beneficial ones (Heinzl, 2022). Moreover, Ventar D's antioxidant and anti-inflammatory activity support better gut barrier functioning. Better gut health leads to higher growth performance and improved feed conversion, which could be demonstrated in several trials with broilers (figures 2 and 3). In the tests, a group fed Ventar D was compared to either a control group with no such feed supplement or groups supplied with competitor products at the recommended inclusion rates.

Compared to a negative control group, the Ventar D group consistently showed a higher average daily gain of 0.3-4.1 g (0.5-8.5 %) and a 3-4 points better feed conversion. Compared to competitor products, Ventar D provided 1-1.7 g (2-3 %) higher average daily gain and a 3 points better /1 point higher FCR than competitors 2 and 1.

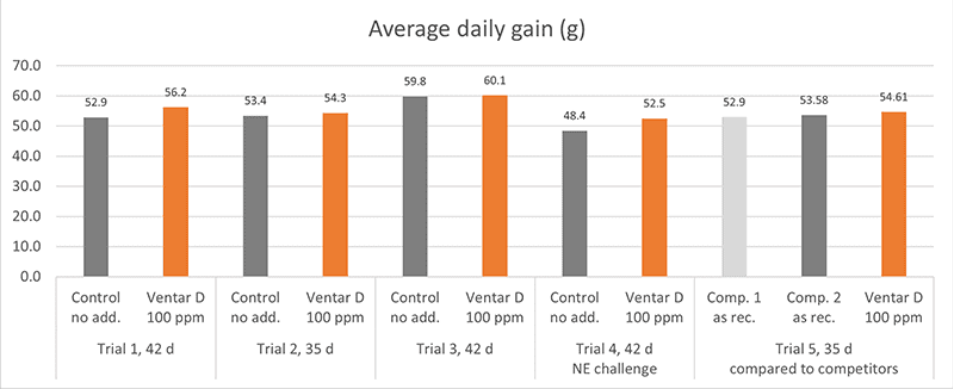

Figure 2: Average daily gain (g) – results of several trials conducted with broilers

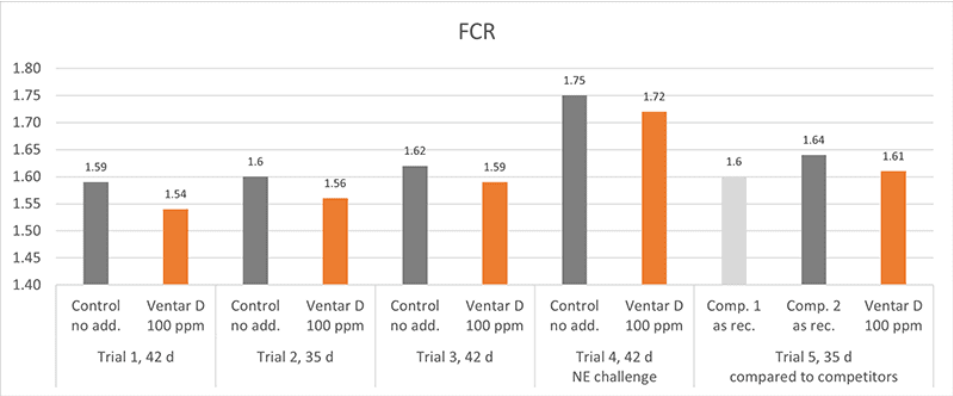

Figure 3: FCR – results of several trials conducted with broilers

Standardization and new technologies for higher profitability

Several in vitro and in vivo studies proved that Ventar D takes "phytomolecules' power" to the next level: Combining standardized phytomolecules and optimal active ingredient protection leads to superior product stability during feed processing. The higher amount of active ingredients arriving in the gut improves gut health and increases the production performance of the animals. Ventar D shows how we can use phytomolecules more effectively and benefit from higher farm profitability.

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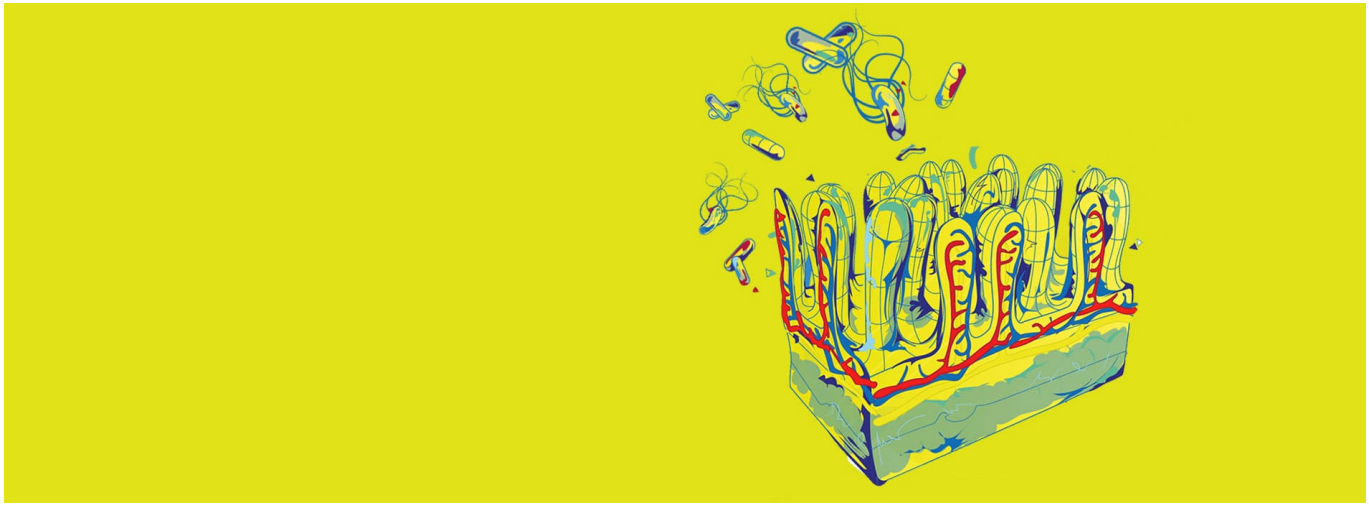
Pushing the microbiome in the right direction – with phytomolecules!



by Dr. Inge Heinzl, Editor

From day 1, young animals are confronted with the pathogens of their environment. Feed and

feed ingredients also significantly increase exposure to microbes. This article will look closely at three critical bacteria in poultry production. The trials of phytomolecules-based products shared in this article prove the unique benefit of lowering harmful pathogens while simultaneously sparing health-promoting microbes. The targeted selection of the blend's phytomolecules contributes to this distinctive mode of action.

E. coli can be valuable… and dangerous

E.coli are commensal bacteria that usually belong to the natural gut flora. However, there are several E. coli strains that, due to certain virulence factors, can cause disease. These bacteria are called avian pathogenic E. coli or APEC. The disease 'Colibacillosis' can occur in different forms:

- Omphalitis – a noncontagious infection of the navel and/or yolk sac in young poultry
- peritonitis – inflammatory response on "internal laying" (yolk material in the peritoneum)
- salpingitis – inflammation of the oviduct
- cellulitis – discoloration and thickening of the skin, inflammation of the subcutaneous tissues
- synovitis – lameness with swollen joints
- coligranuloma (Hjärre disease) – lesions similar to tuberculosis, not of economic importance
- meningitis, and
- septicemia or blood poisoning.

Since some of the *E. coli* strains can sometimes be transmitted vertically to offspring, it is crucial to keep the pathogenic pressure in the parent generation as low as possible (Mc Dougal, 2018).

Due to the, mostly in young chicks, common use of antibiotics, *E. coli* strains resistant to ß-lactam antibiotics (ESBL-producing *E. coli*) or fluoroquinolones (e.g., Enrofloxacin) have developed.

Clostridium perfringens: the cause of necrotic enteritis

Clostridium perfringens belong to the normal caecal flora. However, its overgrowth in the intestine is linked to necrotic enteritis, causing estimated losses of up to USD 6 billion yearly in global poultry production, which corresponds to USD 0.0625 per bird (Wade and Keyburn, 2015). Necrotic enteritis can occur in a clinical and a subclinical form.

In the case of clinical necrotic enteritis, the birds suffer from diarrhea resulting in wet litter and increased flock mortality of up to 1 % per day (Ducatelle and Van Immerseel, 2010). Mortality rates sometimes sum up to 50 % (Van der Sluis, 2013). If birds die without clinical signs, it may be peracute necrotic enteritis.

The subclinical version, however, is more critical. Due to the lack of symptoms, it often remains undetected and, therefore, not treated. Mainly through the impaired utilization of feed, representing 65-75 % of the total costs in broiler production, subclinical necrotic enteritis permanently impacts production efficiency (Heinzl et al., 2020).

Salmonella enterica: a zoonosis relevant for birds and humans

Most concerning in (non-typhoid) salmonellosis is that it can be transferred to humans. The transmission occurs via direct contact with an infected animal, consuming contaminated animal products such as meat or eggs, contact with infected vectors (insects or pets) or contaminated equipment, or cross-contamination in the kitchen. Frozen or raw chicken products, as well as the eggs, are frequent causes of animal-origin Salmonella infections in humans.

Salmonella is the more critical the younger the birds. If the hatching eggs already carry salmonellae, the hatchability dwindles. During their first weeks of life, infected chicks show higher mortality and systemic infections.

Adult animals usually do not die from salmonellosis; often, the infection remains unnoticed. During an acute salmonella outbreak, the animals might show weakness and diarrhea. They lose weight, resulting in decreased egg production in layers.

Trials with phytomolecules show promising results

To check if phytomolecules-based products can effectively influence gut flora, a product specially designed for gut health (Ventar D) was tested for its antimicrobial activity. Additionally, the extent to which the same blend impacted the beneficial bacteria, such as Lactobacilli, was evaluated.

Trial 1: phytomolecules act against *E. coli* and *Salmonella enterica*

The in vitro study using the agar dilution method was conducted at a German laboratory.

The bacteria (*Salmonella typhimurium* and ESBL-producing *E. coli*) stored at -80°C were reactivated by cultivating them on Agar Mueller Hinton overnight. After this incubation, some colonies were picked and suspended in 1 ml 0.9% NaCl solution. 100 µl of the suspension were pipetted and evenly spread (plate spread technique) on new Agar Mueller Hinton containing different concentrations of a phytomolecules-based product (Ventar D): 0 µg/mL – control; 500 µg/mL; 900 µg/mL; 1.250 µg/mL and 2.500 µg/mL. After 16-20 h incubation at 37°C, growth was evaluated. The results can be seen in pictures 1 and 2:

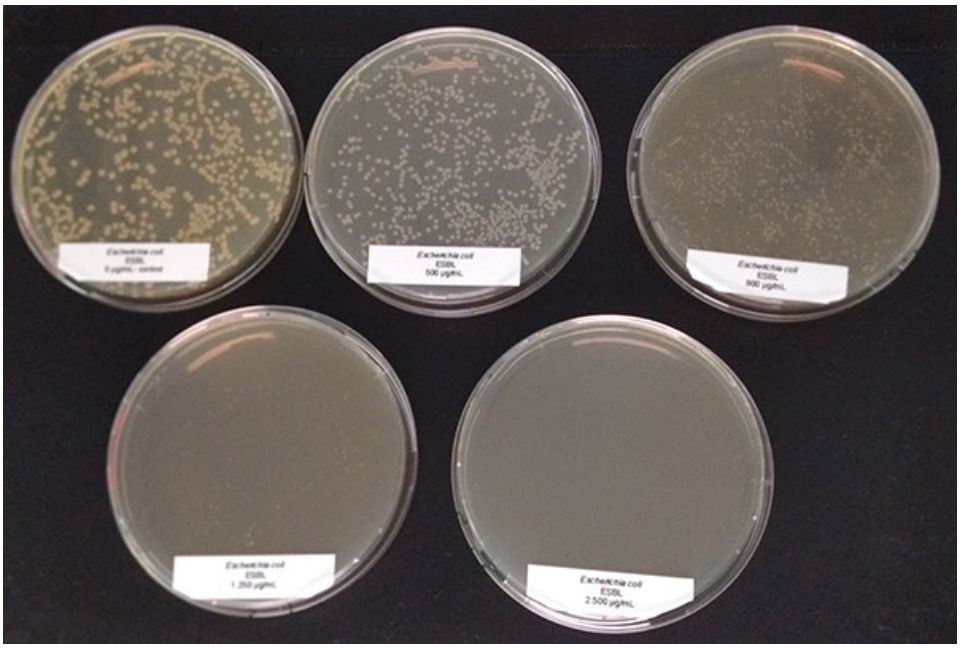


Figure 1: E. coli exposed to different concentrations of Ventar D (upper row from left to right: control 0 µg/ml, 500 µg/ml, 900 µg/ml; lower row from left to right: 1250 µg/ml and 2500 µg/ml)

E. coli colonies exposed to 900 µg/mL of Ventar D's phytogenic formulation were smaller than the control colonies. At 1250 µg/mL, fewer colonies were detected, and at 2500 µg/mL, growth couldn't be seen anymore.

The salmonella colonies showed a similar picture; however, the reduction could be seen from a concentration of 1.250 µg/ml of Ventar D onwards (picture 2).

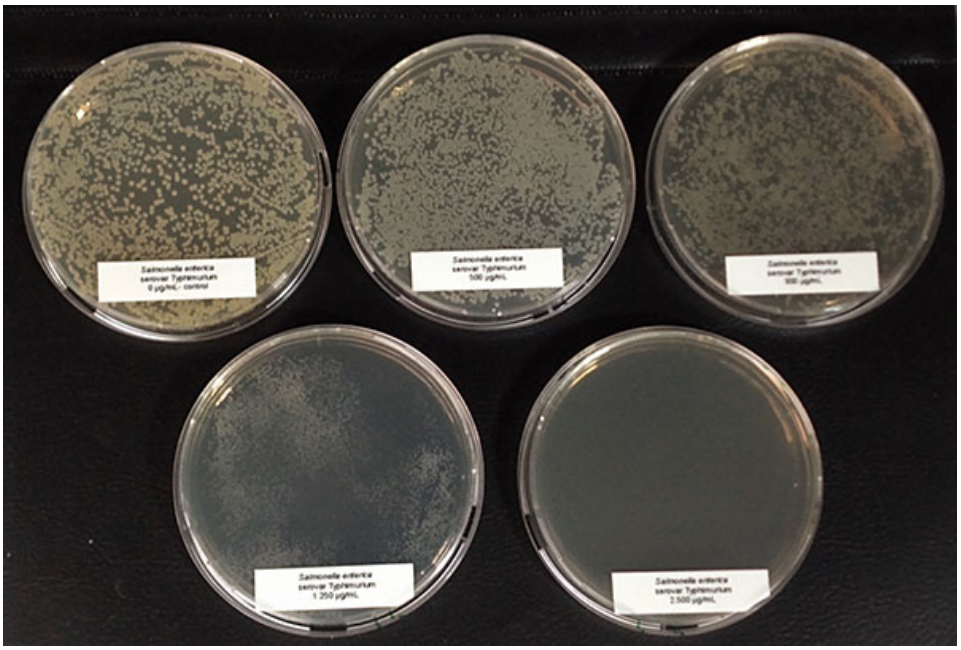


Figure 2: Salmonella enterica exposed to different concentrations of Ventar D (upper row from left to right: control 0 µg/ml, 500 µg/ml, 900 µg/ml; lower row from left to right: 1250 µg/ml and 2500 µg/ml)

Trial 2: Phytomolecules inhibit Clostridium perfringens and spare Lactobacilli

In this trial, the bacteria (*Clostridium perfringens, Lactobacillus agilis S73*, and *Lactobacillus plantarum*) were cultured under favorable conditions (RCM, 37°C, anaerobe for Clostr. perfr., and MRS, 37°C, 5 % CO_2 for Lactobacilli) and exposed to different concentrations of Ventar D (0 µg/ml – control, 500 µg/ml, 750 µg/ml, and 1000 µg/ml).

The results are shown in figures 3a-d.

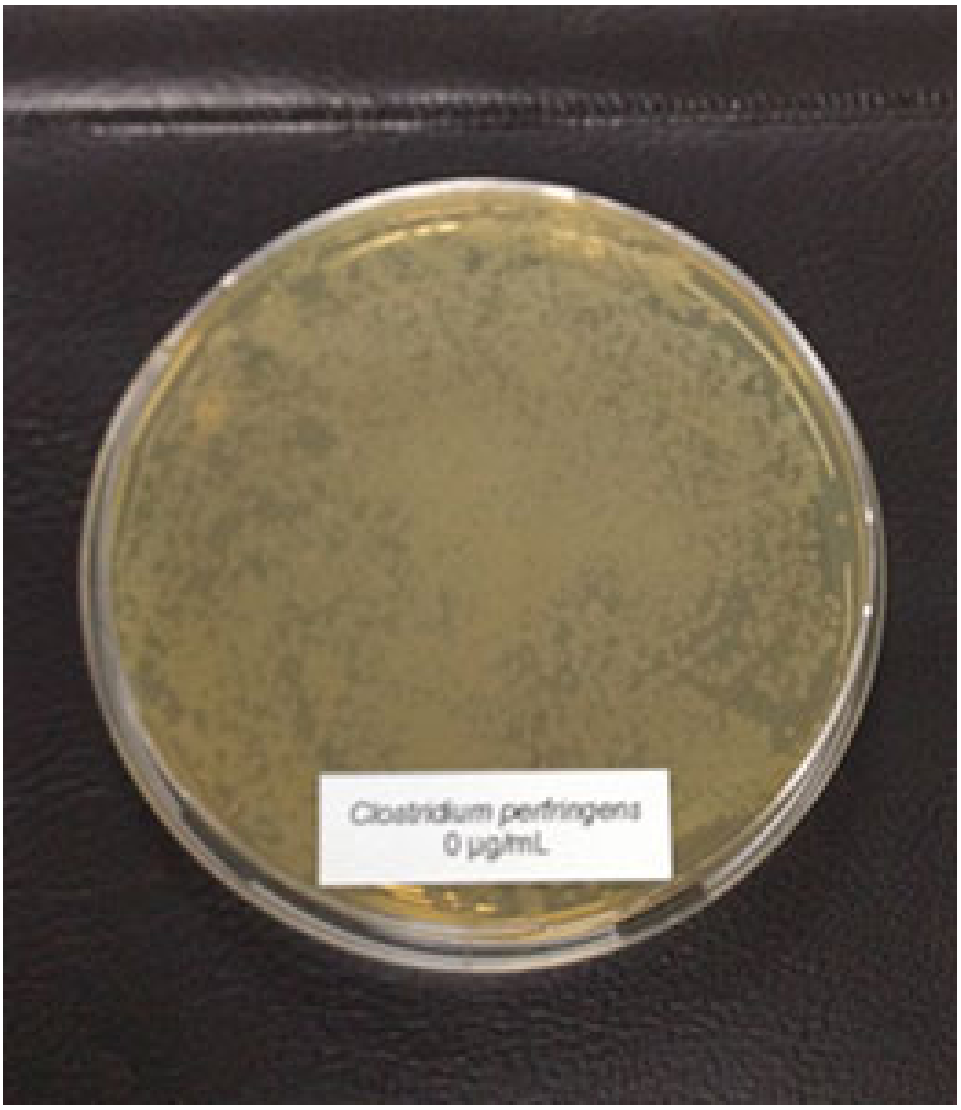


Figure 3a: control, 0 µg/ml

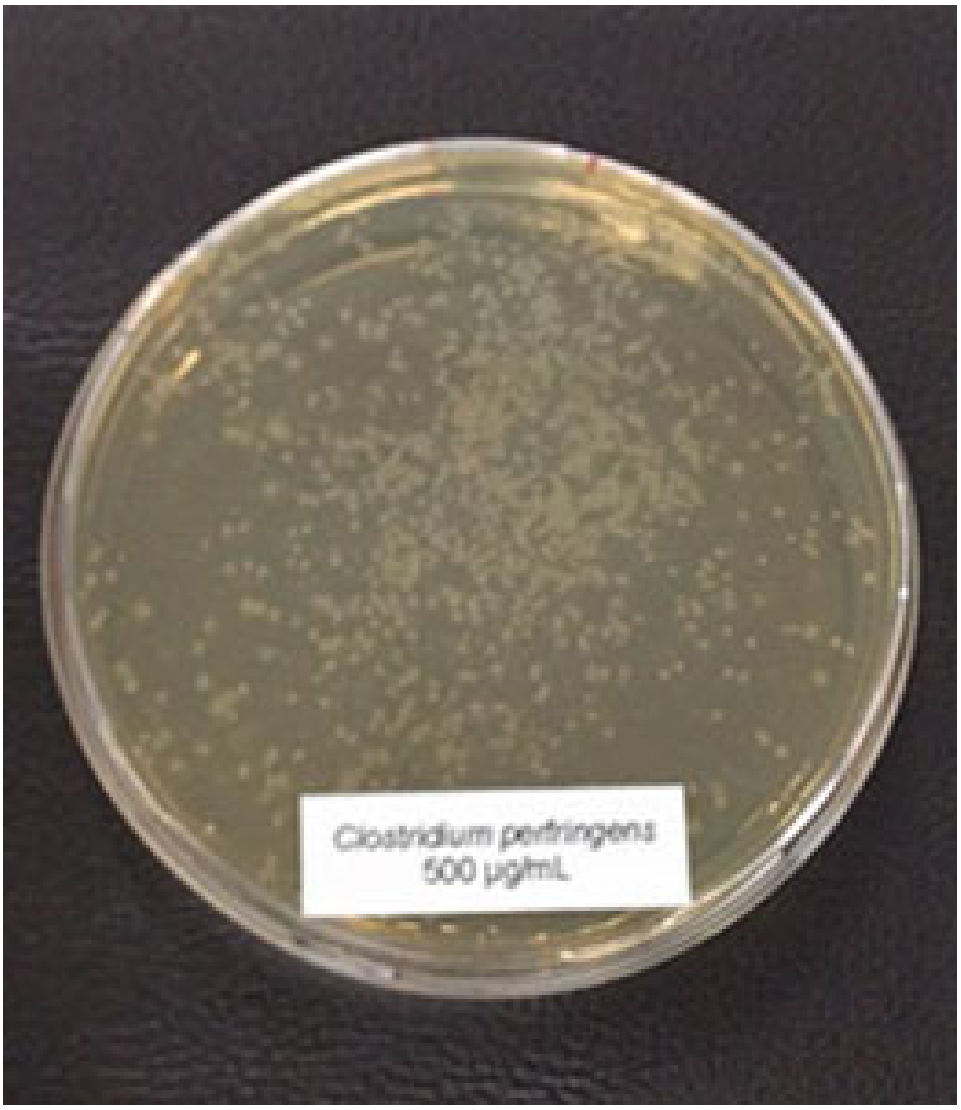


Figure 3b: 500 µg/ml

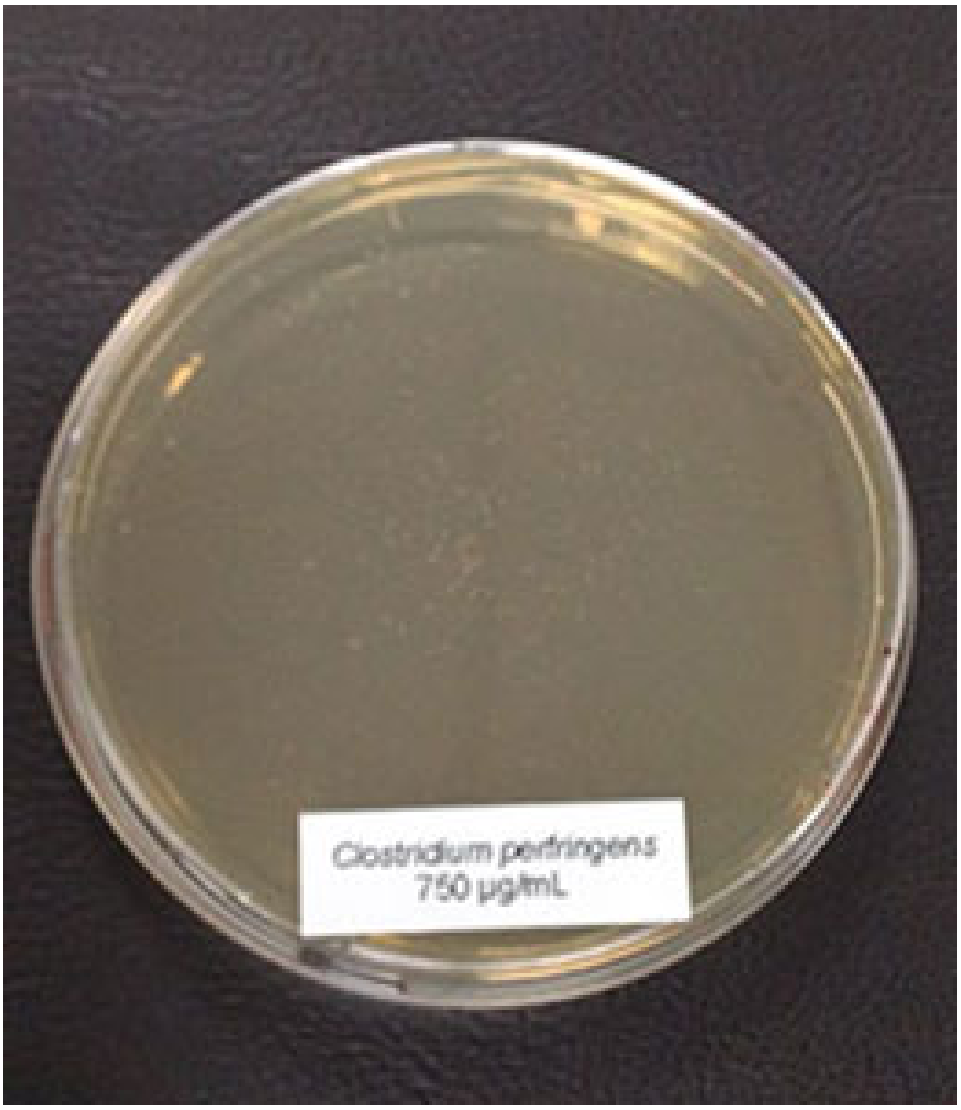


Figure 3c: 750 µg/ml

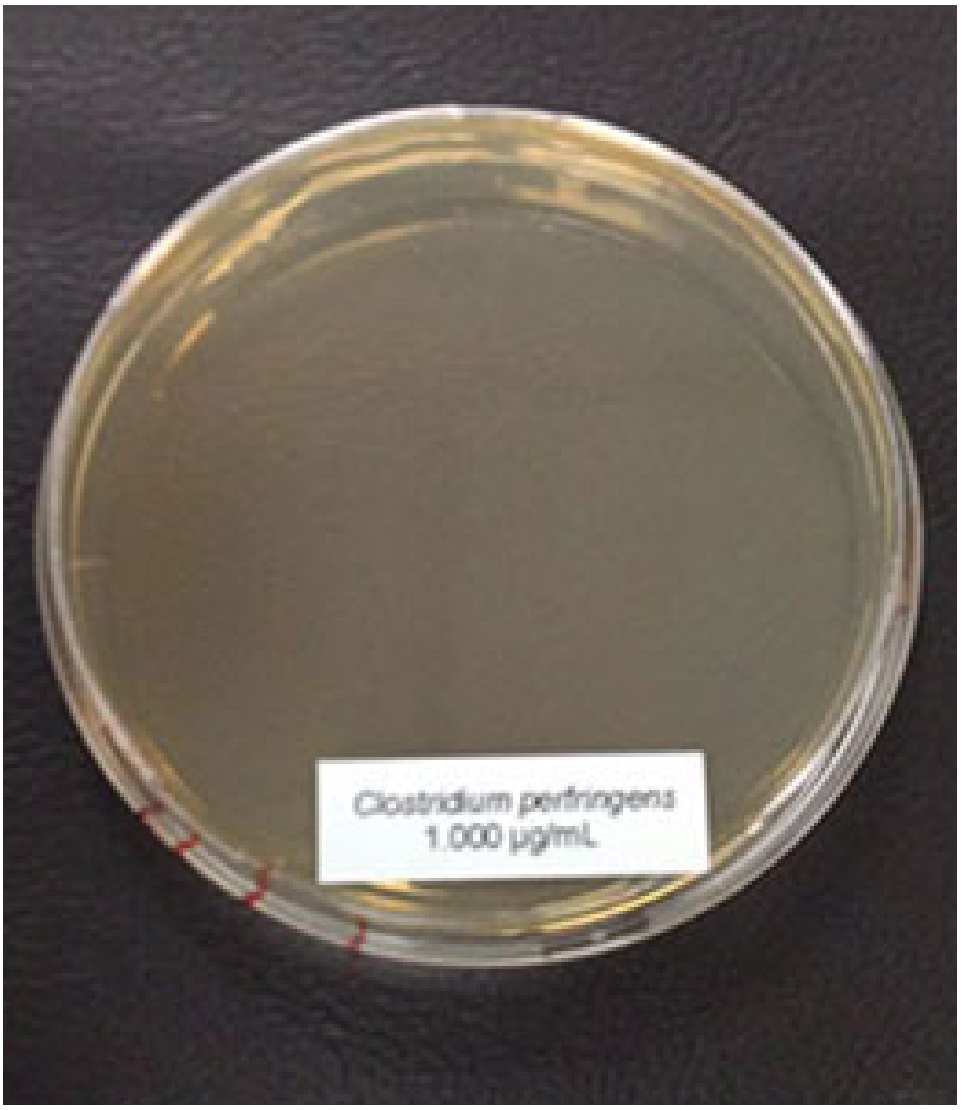


Figure 3d: 1000 µg/m

In the case of Clostridium perfringens, a significant reduction of colonies could already be observed at a concentration of 500 µg/ml of Ventar D. At 750 µg/ml, only a few colonies remained. At a Ventar D concentration of 1000 µg/ml, Clostridium perfringens could no longer grow.

In contrast to Clostridium, the Lactobacilli showed a different picture: only at the higher concentration (1250 µg/ml of Ventar D), Lactobacillus plantarum and Lactobacillus agilis S73 showed a slight growth reduction (figures 4 and 5).

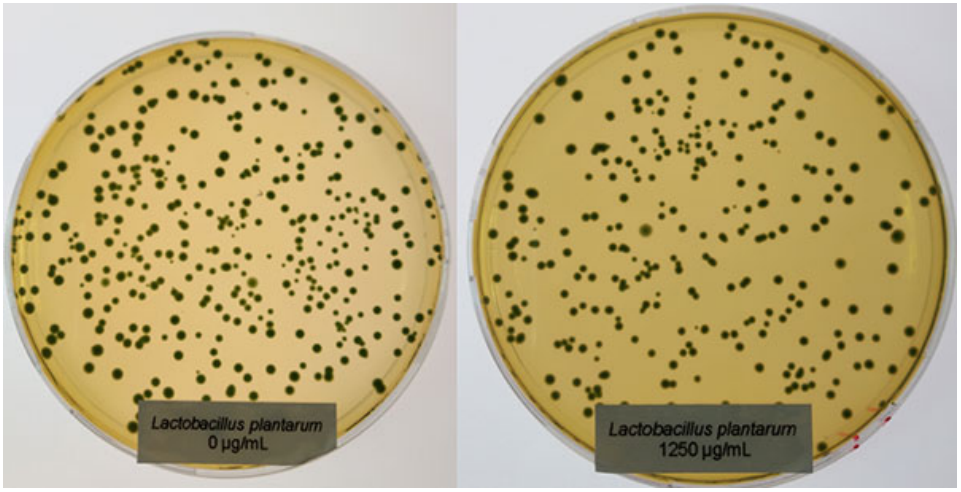


Figure 4: Lactobacillus plantarum exposed to 0 (left) and 1250 µg/ml (right) of Ventar D

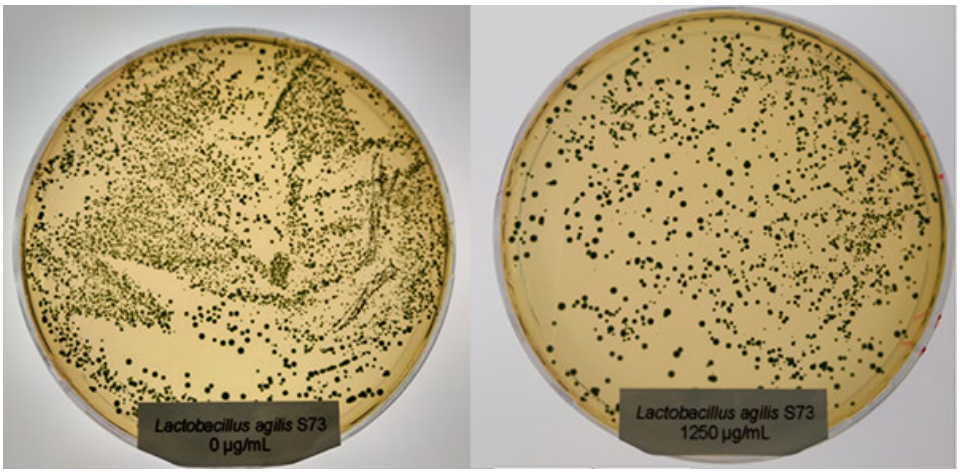


Figure 5: Lactobacillus agilis S73 exposed to 0 (left) and 1250 µg/ml (right) of Ventar D

Improve gut health by positively influencing the intestinal flora

The experiments show that even at lower concentrations, phytomolecules impair the growth of harmful bacteria while sparing the beneficial ones. Phytomolecule-based products can be regarded as a valuable tool for controlling relevant pathogens in poultry and influencing the microflora composition in a positive way.

The resulting better gut health is the best precondition to reducing antibiotics in animal production.