Pathogenic Enterococcus cecorum – an emerging profit killer for broiler producers

*By **Dr. Ajay Bhoyar**, Global Technical Manager, EW Nutrition*

Pathogenic Enterococcus cecorum (EC) is emerging as a significant challenge in poultry production worldwide, causing substantial losses to commercial flocks. EC has become a considerable concern for the poultry industry, not only because of its rapid spread and negative impact on broiler health but also because of its increasing antibiotic resistance. As a result, there is a growing need to explore alternative ways of controlling this bacterium. There is no silver bullet yet as a replacement for antibiotics to limit the load of E. cecorum. Maintaining optimum gut health to avoid E. cecorum leakage during the first week of the broiler's life can control losses due to E. cecorum.

Phytogenic compounds, which are derived from plants, have gained attention in the last decades as a potential solution for controlling common gut pathogens. These natural compounds have been found to possess antimicrobial properties and can help improve gut health in broilers. In this article, we will discuss the current state of E. cecorum and explore potential strategies, including using phytogenic compounds as support in controlling economic losses due to this emerging pathogen in broiler production.

Enterococcus cecorum and its negative impact on broiler production

E. cecorum is a component of normal enterococcal microbiota in the gastrointestinal tract of poultry. These are facultatively anaerobic, gram-positive cocci. Over the past 15 years, pathogenic strains of E. cecorum have emerged as an important cause of skeletal disease in broiler and broiler breeder chickens (Broast et al., 2017; Jackson et al., 2004 and Jung et al., 2017). Along with the commensal strains of EC, the pathogenic strains also occur and can result in Enterococcal spondylitis (ES), also known as "kinky back", a serious disease of commercial poultry production in which the bacteria translocate from the intestine to the free thoracic vertebrae and adjacent notarium or synsacrum, causing lameness, hind-limb paresis and, in 5 to 15% of cases, mortality (de Herdt et al., 2008; Martin et al., 2011; Jung and Rautenschlein, 2014).

The compression of the spinal cord due to infection of the free thoracic vertebra results in the so-called "kinky back" in the skeletal phase of E. cecorum infection. Kinky back is also a common name for spondylolisthesis, a developmental spinal anomaly. EC is normally found in the gastrointestinal tract and may need the help of other factors, such as a leaky gut, to escape the gastrointestinal tract. The emergent pathogenic strains of E. cecorum have developed an array of virulence factors that allow these strains to 1) colonize the gut of birds in the early life period; 2) escape the gut niche; 3) spread systemically while evading the immune system; and 4) colonize the damaged cartilage of the free thoracic vertebra (Borst, 2023). The E. cecorum can invade internal organs and produce lesions in the pericardium, lung, liver, and spleen.

The negative impact of E. cecorum on broiler economics, health, and welfare

Enterococcus cecorum can harm broiler health, welfare, and economics. This can result in decreased profitability for broiler producers.

The broiler flocks infected with E. cecorum may have reduced feed intake/ nutrient absorption and reduced growth rates, leading to a higher feed conversion ratio, longer production cycles, and lower weight gain. The morbidity and mortality from E. cecorum infection can be as high as 35 % and 15%, respectively. The higher condemnations of up to 9.75% at the processing plant can further add to the losses (Jung et al., 2018). This can result in significant economic losses for producers.

Further, E. cecorum infections can impair the immune function of broilers, making them more susceptible to other pathogens and reducing their overall health and welfare. Pathogenic E. cecorum is an opportunistic pathogen that can gain momentum during coinfection with E. coli and other gut pathogens, causing a leaky gut. Therefore, a holistic approach to gut health management may help reduce the losses.

Antibiotic resistance in E. cecorum

E. cecorum has been found to be resistant to multiple antibiotics. Multidrug resistant pathogenic E. cecorum could be recovered from lesions in whole birds for sale at local grocery stores (Suyemoto et al., 2017). Antibiotic resistance can make it difficult to treat and control infections in broilers. This can lead to increased use of multiple antibiotics, which can contribute to the development of antibiotic-resistant bacteria and pose a risk to human health.

Transmission of E. cecorum in broiler flocks

Despite the rapid global emergence of this pathogen, and several works on the subject, the mechanism by which pathogenic E. cecorum spreads within and among vertically integrated broiler production systems remains unclear (Jung et. al.2018). The role of vertical transmission of pathogenic E. cecorum remains elusive. Experimentally infected broiler breeders apparently do not pass the bacterium into their eggs or embryos (Thoefner and Peter, 2016). However, it has been noted that a very low frequency of infected chicks can cause a flock-wide outbreak.

Horizontal transmission: E. cecorum can be transmitted between birds within a flock through direct contact or exposure to contaminated feces, feed, or water.

While the mode of transmission between flocks has not been definitively identified, pathogenic E. cecorum demonstrates rapid horizontal transmission within flocks. It can spread rapidly within flocks via fecal-oral transmission.

Personnel and equipment: E. cecorum can be introduced into a flock through personnel or equipment that has been in contact with infected birds or contaminated materials. For example, personnel working with infected flocks or equipment used in infected flocks can spread the pathogen to uninfected flocks.

Symptoms and diagnosis of E. cecorum in broilers

Enterococcus cecorum infections in broilers can present a range of symptoms, from mild to severe. The most common symptom noticed with E. cecorum is paralysis, which is due to an inflammatory mass that develops in the spinal column at the level of the free thoracic vertebra (FTV). Recognition of this spinal lesion has given rise to several disease names for pathogenic E. cecorum infection, which include vertebral osteomyelitis, vertebral enterococcal osteomyelitis and arthritis, enterococcal spondylitis (ES), spondylolisthesis and, colloquially, “kinky-back” (Jung et al. 2018).

E. cecorum infections can exhibit increased mortality due to septicemia in the early growing period. In this sepsis phase, the clinical signs of E. cecorum may include fibrinous pericarditis, perihepatitis, and air-sacculitis. These lesions might be confused with other systemic bacterial infections like colibacillosis. Therefore, a pure culture is needed for the correct diagnosis of E. cecorum.

The second phase of mortality due to dehydration and starvation of the paralyzed birds can be observed during the finisher phase peaking during 5-6 weeks of age. Paralysis from infection of the free thoracic vertebra is the most striking feature of this disease, with affected birds exhibiting a classic sitting position with both legs extended cranially (Brost et al., 2017).

Diagnosis of E. cecorum in broilers can be challenging, as the symptoms of infection can be similar to those of other bacterial or viral infections. However, a combination of clinical signs, post-mortem examination, and laboratory testing can help to confirm the presence of E. cecorum. Laboratory tests such as bacterial culture and polymerase chain reaction (PCR) can be used to identify the pathogen and also to determine its antibiotic susceptibility. Veterinarians and poultry health professionals can work with producers to develop a diagnostic plan and implement appropriate control measures to manage E. cecorum infections in broiler flocks.

Prevention and Control of Enterococcus cecorum

The broiler producers/ managers should work with their veterinarians and poultry health professionals to develop an integrated approach to control the spread of E. cecorum and prevent its negative impact on broiler health and productivity.

Currently, there is no commercial vaccine available for preventing pathogenic E. cecorum infection. Therefore, controlling Enterococcus cecorum infection in broiler flocks requires a multifaceted approach that addresses the various modes of transmission and bacterial resistance to antibiotics.

Implementing strict biosecurity protocols, such as controlling access to the farm, disinfecting equipment and facilities, and implementing proper hygiene protocols throughout the integrated broiler operation, can help to minimize the risk of transmission.

Thorough washing of trays and chick boxes in the hatchery with hot water (60-62°C) mixed with an effective disinfectant can reduce the possible vertical transmission of E. cecorum. The vertical transmission may also be prevented by adopting the practice of separating the dirty floor eggs from clean hatching eggs and setting them in the lower racks of the incubator.

Generally, pathogenic isolates from poultry were found to be significantly more drug-resistant than commensal strains (Borst et al., 2012). The selection of an effective antibiotic for the treatment of E. cecorum should be made based on the results of the antibiotic sensitivity test. Antibiotic therapy may not help with paralyzed birds, which ultimately need to be culled. Reducing the use of antibiotics and implementing prudent use practices can help to reduce the development of antibiotic resistance in E. cecorum and other bacteria.

Probiotics can help to maintain the balance of the gut microbiota and may have a protective effect against E. cecorum infections. Fernandez et al. (2019) reported the inhibitory activities of proprietary poultry Bacillus strains against pathogenic isolates of E. cecorum in vitro, but effects are highly strain-dependent and vary significantly among different pathogenic isolates.

Phytogenic compounds and organic acids have been shown to have antimicrobial properties. Phytomolecule-based preparations may help to control E. cecorum infections in broiler flocks in the first week of life, reducing the chances of its translocation from the intestine.

Phytomolecules-based liquid formulations for on-farm drinking water application can also be a handy tool to manage gut health challenges, especially during risk periods in the life of broilers. Such liquid phytomolecule preparations can help to quickly achieve the desired concentration of the active ingredients for a faster antimicrobial effect.

However, these alternatives to antibiotics may be effective only when the E. cecorum is still localized within the gut during the first two weeks of the broiler chicken’s life.

Phytomolecules, also known as phytochemicals, are naturally occurring plant compounds that have been found to have antimicrobial properties. Especially for commercial poultry, nutraceuticals such as phytochemicals showed promising effects, improving the intestinal microbial balance, metabolism, and integrity of the gut due to their antioxidant, anti-inflammatory, immune modulating, and bactericidal properties (Estevez, 2015). Phytogenic compounds have been studied for their potential use in controlling gut pathogens in poultry. Here are some of the roles that phytomolecules can play in controlling gut pathogens:

Antimicrobial activity: Several phytomolecules, such as essential oils, flavonoids, and tannins, have been found to have antimicrobial activity. Hovorková et al (2018) studied the inhibitory effects of hydrolyzed plant oils (palm, red palm, palm kernel, coconut, babassu, murumuru, tucuma, and Cuphea oil) containing medium-chain fatty acids (MCFAs) against Gram-positive pathogenic and beneficial bacteria. They concluded that all the hydrolyzed oils were active against all tested bacteria (Clostridium perfringens, Enterococcus cecorum, Listeria monocytogenes, and Staphylococcus aureus), at 0.14–4.5 mg/ml, the same oils did not show any effect on commensal bacteria (Bifidobacterium spp. and Lactobacillus spp.). However, further research is needed to test the in-vivo efficacy of phytogenic compounds against pathogenic E. cecorum infections in poultry.

Anti-inflammatory activity: The other coinfecting gut pathogens of E. cecorum can cause inflammation in the intestinal tract of poultry. This can lead to reduced feed intake and growth. Some phytomolecules have been found to have anti-inflammatory activity and can reduce the severity of inflammation. Capsaicin, a naturally occurring bioactive compound in chili peppers, was found to have antioxidant and anti-inflammatory activity. The tendency of capsaicin to substantially diminish the release of COX-2 mRNA is thought to be the reason for its anti-inflammatory effects (Liu et al., 2021). Thyme oil reduced the synthesis and gene expression of TNF-α, IL-1B, and IL-6 in activated macrophages in a dose-dependent manner, with upregulation of IL-10 secretion (Osana and Reglero, 2012). Cinnamaldehyde has been shown to decrease the expression of several cytokines, such as IL-1 β, IL-6, and TNF-α, as well as iNOS and COX-2, in in-vitro studies (Pannee et al., 2014).

Antioxidant activity: Oxidative stress may contribute to the development of E. cecorum infections in poultry. Phytomolecules, such as polyphenols and carotenoids, have been found to have antioxidant activity and can reduce oxidative stress in the gut of poultry, which can help to prevent E. cecorum infections. Polyphenols widely exist in a variety of plants and have been used for various purposes because of their strong antioxidant ability (Crozier et al., 2009). Quercetin, a flavonoid compound widely present in vegetables and fruits, is well-known for its potent antioxidant effects (Saeed et al., 2017).

Phytomolecules can also modulate the immune system of poultry, which can help to prevent E. cecorum infections. For example, some flavonoids and polysaccharides have been found to enhance the immune response of poultry. Fahnani et. al. (2019) found that supplementing broiler chickens with a combination of flavonoids and polysaccharides extracted from the mushroom *Agaricus blazei* enhanced their immune response.

Overall, phytomolecules have shown promise in supporting the optimum gut health of poultry. Many phytogenic preparations available in the market can be regarded as an important tool to reduce the use of antibiotics in animal production and mitigate the risk of antimicrobial resistance. However, more research is needed to develop an effective combination of active ingredients, as well as strategies for their use in controlling E. cecorum infections in poultry.

Conclusion

In conclusion, the emergence of pathogenic strains of E. cecorum is becoming a major concern for broiler producers globally. This bacterial pathogen can cause significant economic losses in the broiler industry by affecting the overall health and productivity of the birds. Pathogenic E. cecorum infection can lead to clinical signs including diarrhea, decreased feed intake, reduced growth rate, and increased mortality. Proactive measures must be taken to prevent the introduction and spread of pathogenic E. cecorum in broiler flocks. Implementing strict biosecurity protocols and proper disinfection procedures can help reduce the risk of E. cecorum infection. The use of effective antibiotics after receiving the results of the antibiotics sensitivity test is a crucial step in controlling the infection. Phytomolecule-based preparations can be a potential alternative to control the load of E. cecorum by maintaining optimum gut health to minimize economic losses. Moreover, ongoing surveillance and monitoring of pathogenic E. cecorum prevalence in the broiler industry can assist in the timely detection and control of outbreaks.

In summary, the emergence of pathogenic E. Cecorum as a profit killer in the broiler industry warrants careful attention and proactive management practices to minimize its impact.

References:

Borst, L. B., M. M. Suyemoto, A. H. Sarsour, M. C. Harris, M. P. Martin, J. D. Strickland, E. O. Oviedo, and H. J. Barnes. “Pathogenesis of Enterococcal Spondylitis Caused by Enterococcus cecorum in Broiler Chickens”. Vet. Pathol. 54:61-73. 2017.

Crozier A., Jaganath I.B., Clifford M.N. “Dietary phenolics: Chemistry, bioavailability and effects on health”. Nat. Prod. Rep. 2009;26:1001–1043.

De Herdt, P., P. Defoort, J. Van Steelant, H. Swam, L. Tanghe, S. Van Goethem, and M.

593 Vanrobaeys. “Enterococcus cecorum osteomyelitis and arthritis in broiler chickens”. Vlaams Diergeneeskundig Tijdschrift 78:44-48. 2009.

Estévez, M. “Oxidative damage to poultry: From farm to fork”. Poult. Sci. 2015, 94, 1368–1378.

Fanhani, Jamile & Murakami, Alice & Guerra, Ana & Nascimento, Guilherme & Pedroso, Raíssa & Alves, Marília. (2016). “Agaricus blazei in the diet of broiler chickens on immunity, serum parameters and antioxidant activity”. Semina: Ciencias Agrarias. 37. 2235-2246.

Hovorková P., Laloučková K., Skřivanová E. (2018): “Determination of in vitro antibacterial activity of plant oils containing medium-chain fatty acids against Gram-positive pathogenic and gut commensal bacteria”. Czech J. Anim. Sci., 63: 119-125.

Jung, A., and S. Rautenschlein. “Comprehensive report of an Enterococcus cecorum infection in a broiler flock in Northern Germany. BMC Vet. Res. 10:311. 2014.

Jung, A., M. Metzner, and M. Ryll. “Comparison of pathogenic and non-pathogenic Enterococcus cecorum strains from different animal species”. BMC Microbiol. 17:33. 2017.

Jung, Arne, Laura R. Chen, M. Mitsu Suyemoto, H. John Barnes, and Luke B. Borst. “A Review of Enterococcus Cecorum Infection in Poultry.” Avian Diseases 62, no. 3 (2018): 261–71.

Liu, S.J.; Wang, J.; He, T.F.; Liu, H.S.; Piao, X.S. “Effects of natural capsicum extract on growth performance, nutrient utilization, antioxidant status, immune function, and meat quality in broilers”. Poult. Sci. 2021, 100, 101301.

Martin, L. T., M. P. Martin, and H. J. Barnes. “Experimental reproduction of enterococcal spondylitis in male broiler breeder chickens”. Avian Dis. 55:273-278. 2011.

Ocaña, A.; Reglero, G. “Effects of thyme extract oils (from Thymus Vulgaris, Thymus Zygis and Thymus hyemalis) on cytokine production and gene expression of OxLDL-stimulated THP-1-macrophages”. J. Obes. 2012, 2012, 104706.

Pannee C, Chandhanee I, Wacharee L. “Antiinflammatory effects of essential oil from the leaves of Cinnamomum cassia and cinnamaldehyde on lipopolysaccharide-stimulated J774A.1 cells”. J Adv Pharm Technol

Res. 2014 Oct;5(4):164-70.

Saeed, M.; Naveed, M.; Arain, M.A.; Arif, M.; Abd El-Hack, M.E.; Alagawany, M.; Siyal, F.A.; Soomro, R.N.; Sun, C. “Quercetin: Nutritional and beneficial effects in poultry”. World’s Poult. Sci. J. 2017, 73, 355–364.

Suyemoto, M. M., H. J. Barnes, and L. B. Borst. “Culture methods impact recovery of antibiotic-resistant Enterococci including Enterococcus cecorum from pre- and postharvest chicken”. Lett. Appl. Microbiol. 64:210-216. 2017.

Thoefner, I. C., Jens Peter. Investigation of the pathogenesis of Enterococcus cecorum after 736 intravenous, intratracheal or oral experimental infections of broilers and broiler breeders. In: 737 VETPATH. Prato, Italy. 2016.

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

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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.

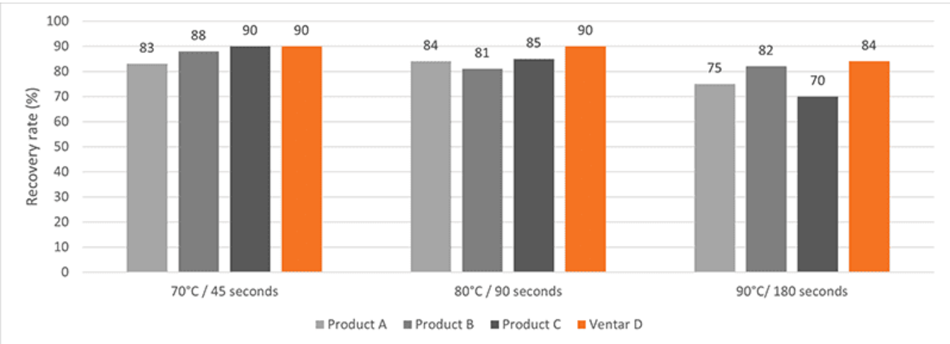


Figure 1: Phytomolecule stability under processing conditions, relative to mash baseline (100%)

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.

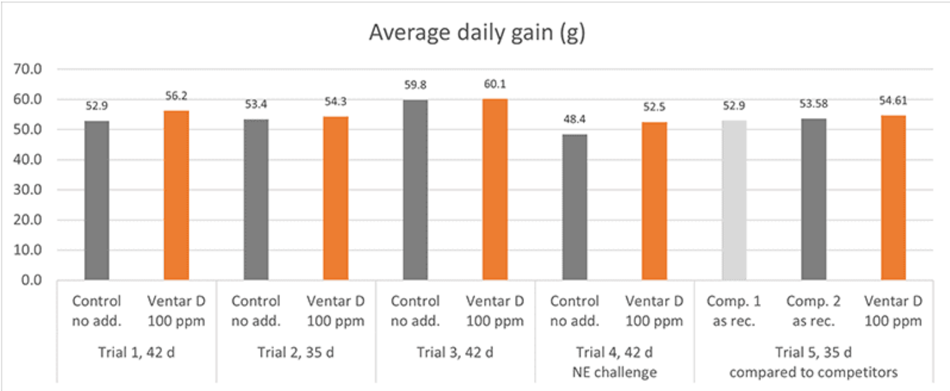


Figure 2: Average daily gain (g) – results of several trials conducted with broilers

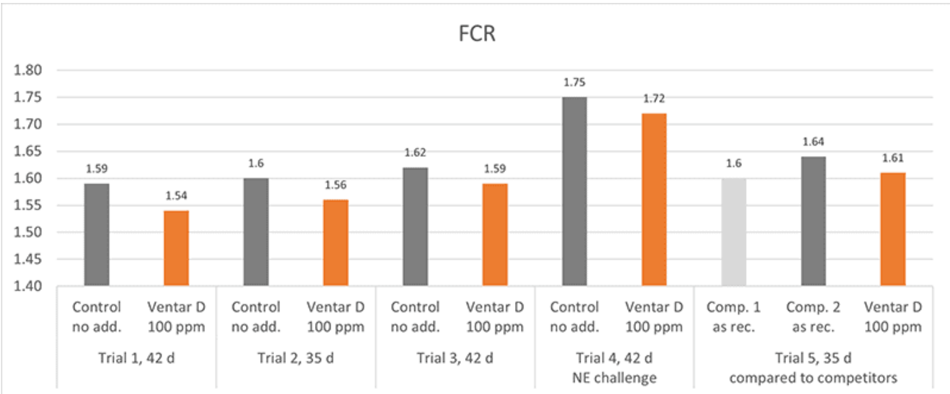


Figure 3: FCR – results of several trials conducted with broilers

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.

References:

Cho, J. H., Y. J. Chen, B. J. Min, H. J. Kim, O. S. Kwon, K. S. Shon, I. H. Kim, S. J. Kim, and A. Asamer. "Effects of Essential Oils Supplementation on Growth Performance, IGG Concentration and Fecal Noxious Gas Concentration of Weaned Pigs". *Asian-Australasian Journal of Animal Sciences* 19, no. 1 (2005): 80–85. https://doi.org/10.5713/ajas.2006.80.

Cuppett, Susan L., and Clifford A. Hall. "Antioxidant Activity of the Labiatae". *Advances in Food and Nutrition Research* 42 (1998): 245–71. https://doi.org/10.1016/s1043-4526(08)60097-2.

Ehrlinger, M. "Phytogenic Additives in Animal Nutrition." Dissertation, Veterinary Faculty of the Ludwig Maximilians University, 2007.

Heinzl, I. "Efficient Microbiome Modulation with Phytomolecules". EW Nutrition, August 30, 2022.

https://ew-nutrition.com/pushing-microbiome-in-right-direction-phytomolecules/.

Hernández, F., J. Madrid, V. García, J. Orengo, and M.D. Megías. “Influence of Two Plant Extracts on Broilers Performance, Digestibility, and Digestive Organ Size.” *Poultry Science* 83, no. 2 (2004): 169–74. https://doi.org/10.1093/ps/83.2.169.

Kowalczyk, Adam, Martyna Przychodna, Sylwia Sopata, Agnieszka Bodalska, and Izabela Fecka. “Thymol and Thyme Essential Oil—New Insights into Selected Therapeutic Applications.” *Molecules* 25, no. 18 (2020): 4125. https://doi.org/10.3390/molecules25184125.

Lindner, , U. “Aromatic Plants – Cultivation and Use.” Düsseldorf: Teaching and Research Institute for Horticulture Auweiler-Friesdorf, 1987.

Oetting, Liliana Lotufo, Carlos Eduardo Utiyama, Pedro Agostinho Giani, Urbano dos Ruiz, and Valdomiro Shigueru Miyada. “Efeitos De Extratos Vegetais e Antimicrobianos Sobre a Digestibilidade Aparente, O Desempenho, a Morfometria Dos Órgãos e a Histologia Intestinal De Leitões Recém-Desmamados.” *Revista Brasileira de Zootecnia* 35, no. 4 (2006): 1389–97. https://doi.org/10.1590/s1516-35982006000500019.

Sadeh, Dganit, Nadav Nitzan, David Chaimovitsh, Alona Shachter, Murad Ghanim, and Nativ Dudai. “Interactive Effects of Genotype, Seasonality and Extraction Method on Chemical Compositions and Yield of Essential Oil from Rosemary (Rosmarinus Officinalis L”.).” *Industrial Crops and Products* 138 (2019): 111419. https://doi.org/10.1016/j.indcrop.2019.05.068.

Shabnum, Shazia, and Muzafar G. Wagay. “Essential Oil Composition of Thymus Vulgaris L. and Their Uses”. *Journal of Research & Development* 11 (2011): 83–94.

Sienkiewicz, Monika, Monika Łysakowska, Marta Pastuszka, Wojciech Bienias, and Edward Kowalczyk. “The Potential of Use Basil and Rosemary Essential Oils as Effective Antibacterial Agents.” *Molecules* 18, no. 8 (2013): 9334–51. https://doi.org/10.3390/molecules18089334.

Smith-Palmer, A., J. Stewart, and L. Fyfe. “Antimicrobial Properties of Plant Essential Oils and Essences against Five Important Food-Borne Pathogens”. *Letters in Applied Microbiology* 26, no. 2 (1998): 118–22. https://doi.org/10.1046/j.1472-765x.1998.00303.x.

Soković, Marina, Jelena Vukojević, Petar Marin, Dejan Brkić, Vlatka Vajs, and Leo Van Griensven. “Chemical Composition of Essential Oils of Thymus and Mentha Species and Their Antifungal Activities”. *Molecules* 14, no. 1 (2009): 238–49. https://doi.org/10.3390/molecules14010238.

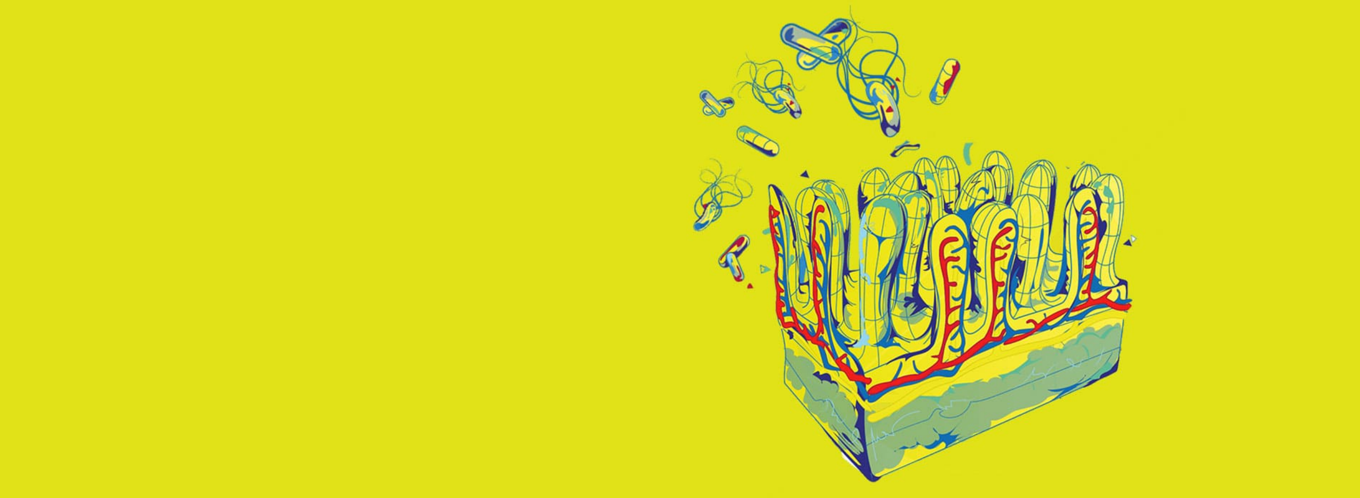
Wei, Alfreda, and Takayuki Shibamoto. “Antioxidant Activities and Volatile Constituents of Various Essential Oils.” *Journal of Agricultural and Food Chemistry* 55, no. 5 (2007): 1737–42. https://doi.org/10.1021/jf062959x.

Yang, Li, Kui-Shan Wen, Xiao Ruan, Ying-Xian Zhao, Feng Wei, and Qiang Wang. “Response of Plant Secondary Metabolites to Environmental Factors”. *Molecules* 23, no. 4 (2018): 762. https://doi.org/10.3390/molecules23040762.

Özer, Hakan, Münevver Sökmen, Medine Güllüce, Ahmet Adigüzel, Fikrettin Şahin, Atalay Sökmen, Hamdullah Kiliç, and Özlem Bariş. “Chemical Composition and Antimicrobial and Antioxidant Activities of the Essential Oil and Methanol Extract of *Hippomarathrum Microcarpum* (Bieb.) from Turkey”. *Journal of Agricultural and Food Chemistry* 55, no. 3 (2007): 937–42. https://doi.org/10.1021/jf0624244.

Efficient microbiome modulation

with phytomolecules



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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:

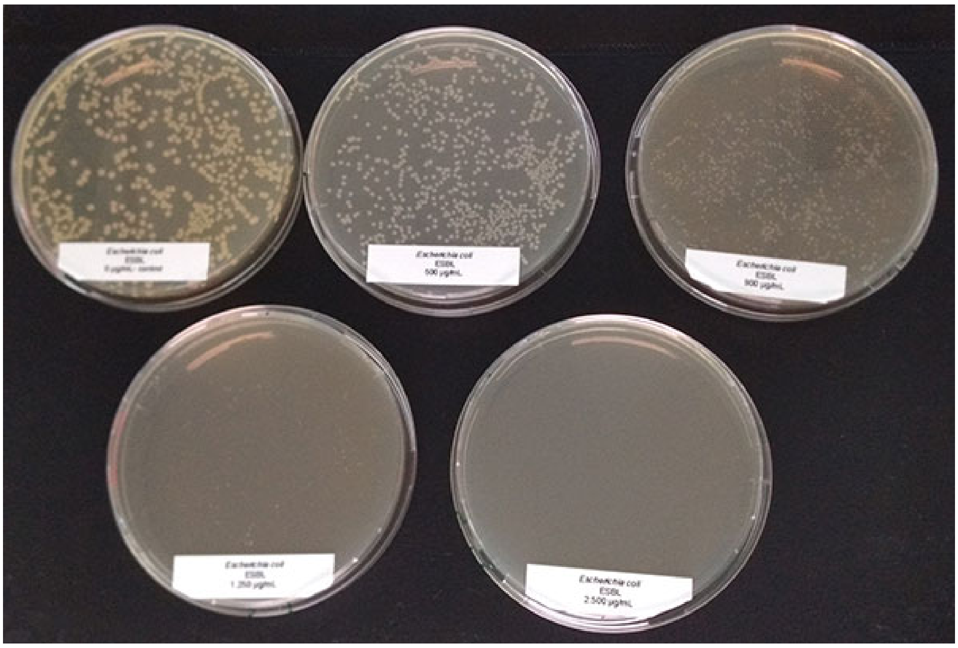

Figure 1: E. coli exposed to different concentrations of Ventar D (upper row from left to right: control 0 µg/ml, 500 µg/ml, 900 µg/ml; lower row from left to right: 1250 µg/ml and 2500 µg/ml)

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).

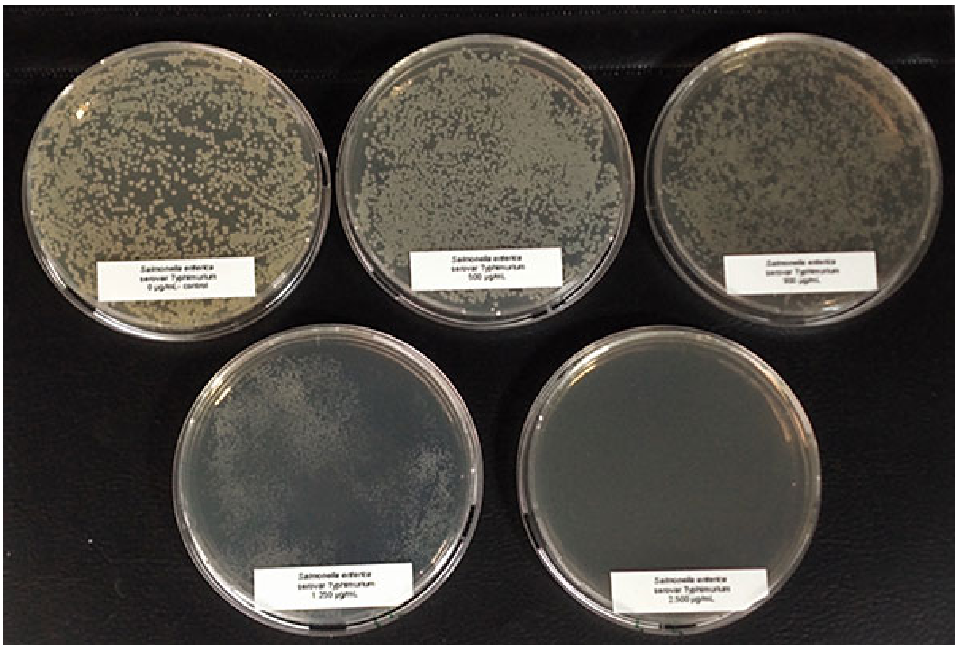

Figure 2: Salmonella enterica exposed to different concentrations of Ventar D (upper row from left to right: control 0 µg/ml, 500 µg/ml, 900 µg/ml; lower row from left to right: 1250 µg/ml and 2500 µg/ml)

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.

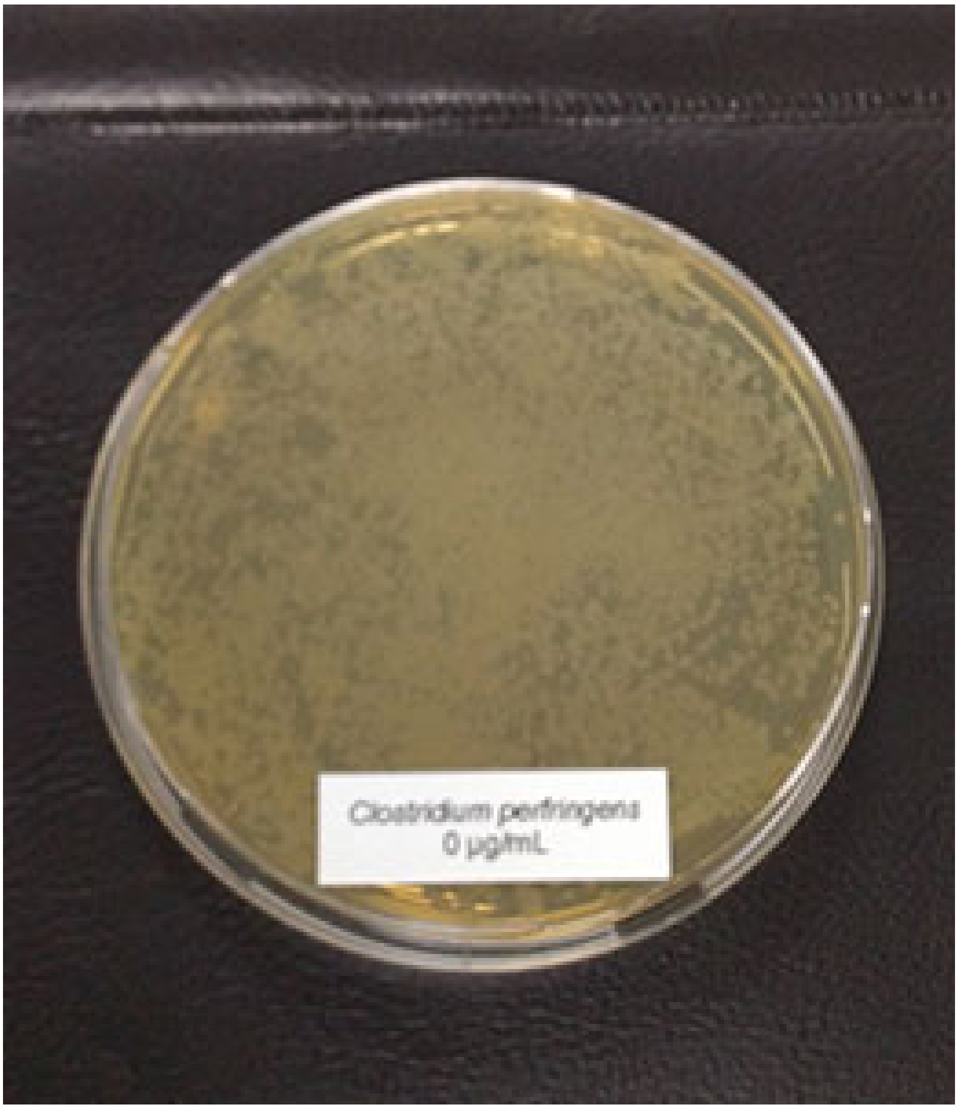

Figure 3a: control, 0 µg/ml

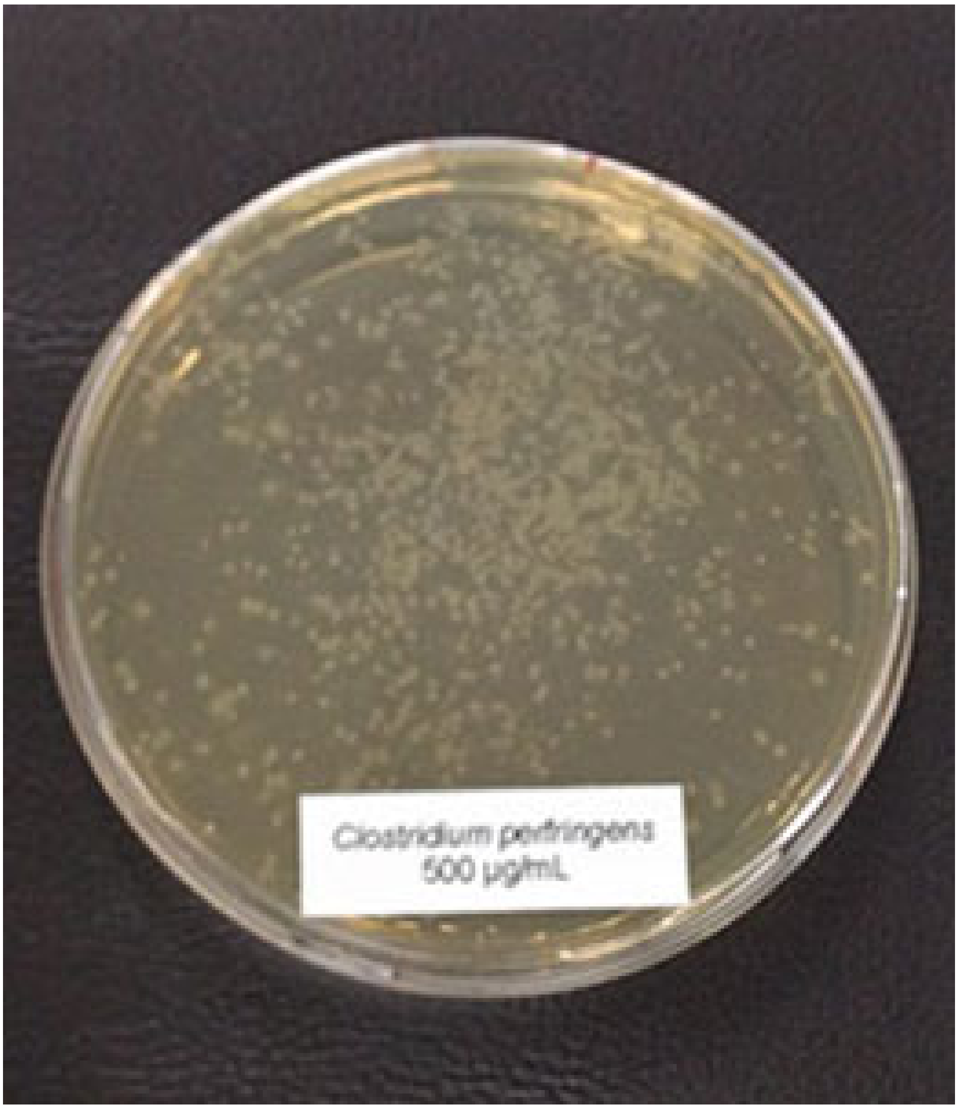

Figure 3b: 500 µg/ml

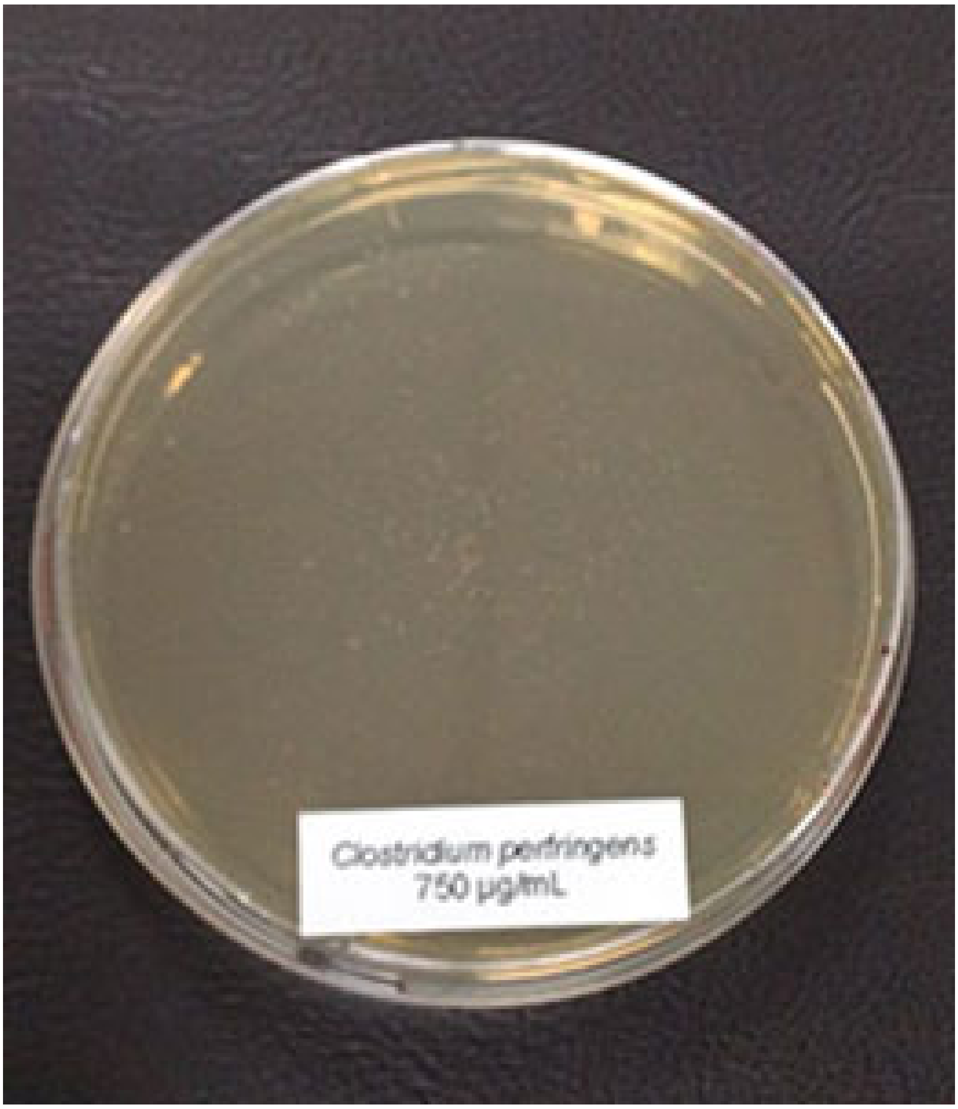

Figure 3c: 750 µg/ml

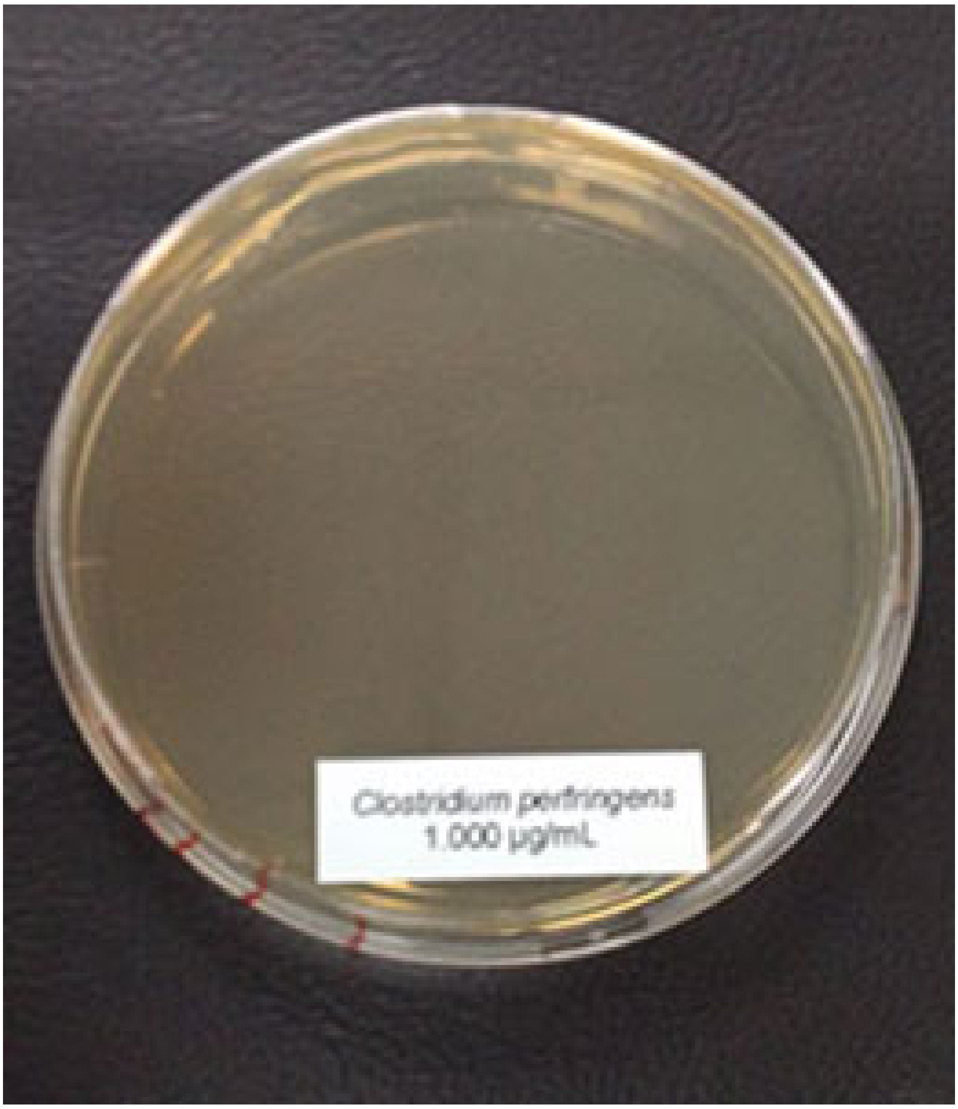

Figure 3d: 1000 µg/m

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).

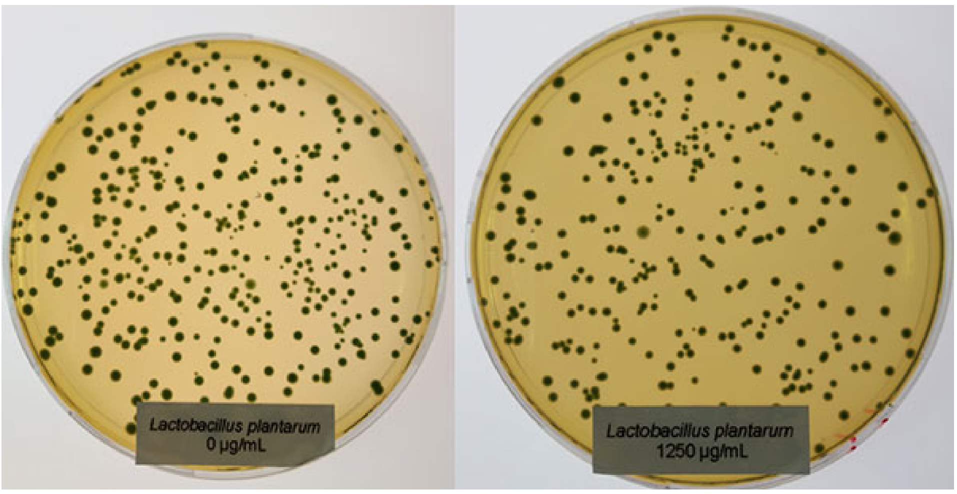

Figure 4: Lactobacillus plantarum exposed to 0 (left) and 1250 µg/ml (right) of Ventar D

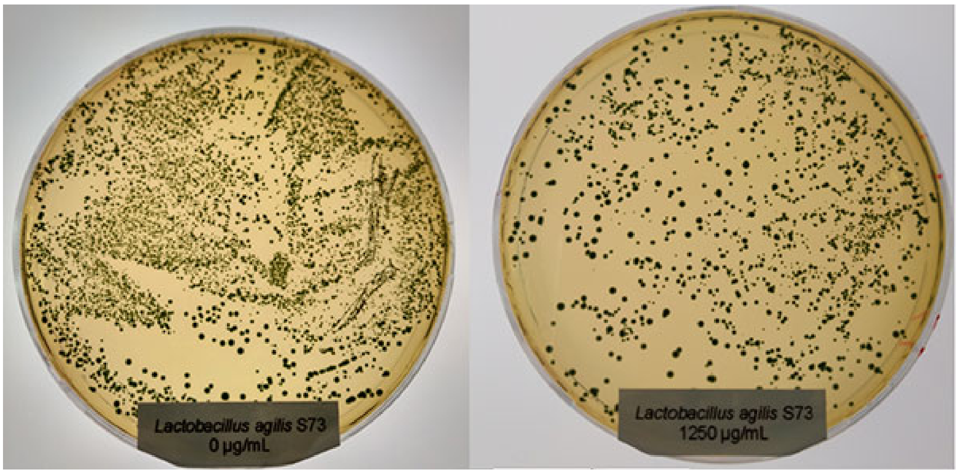

Figure 5: Lactobacillus agilis S73 exposed to 0 (left) and 1250 µg/ml (right) of Ventar D

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.