

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.

Secondary plant compounds are the new frontier in poultry nutrition



Why should you read another story about phyto-genics? 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 feds, 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.

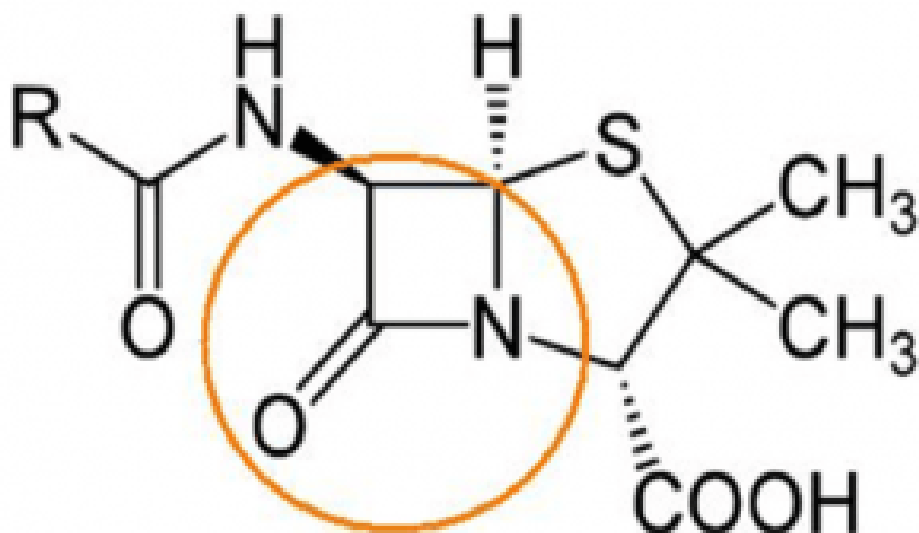
Secondary plant compounds against antibiotic-resistant *E. coli*



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

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

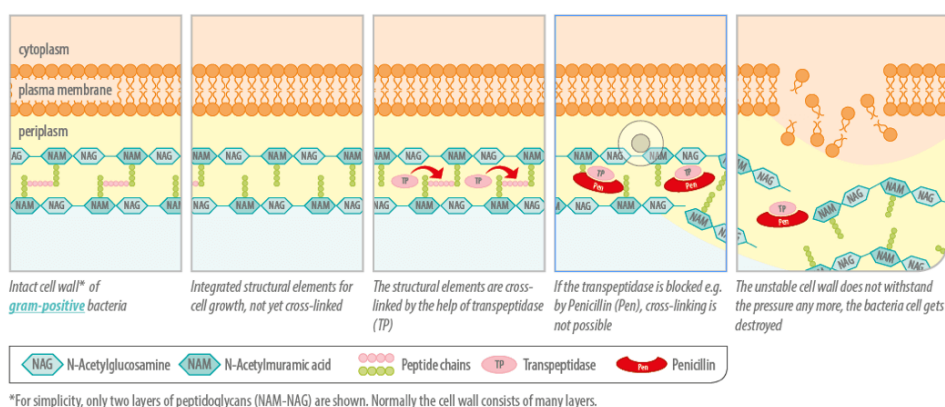


Mode of action of β -lactam antibiotic

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. β -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 β -lactamases, destroy the β -lactam antibiotics and prevent their own destruction.

Secondary plant compounds

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



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

Trial (Scotland)

Design

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

Results

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

Phytogenics can positively influence the efficacy of antibiotics



Many veterinary antibiotics are applied via the waterline, [where they are dosed in combination with other feed additives](#). Amongst those are mixtures of secondary plant compounds with a proven antimicrobial efficacy against veterinary pathogenic bacteria. However, little research has been done to evaluate any effect that antibiotics and 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, β -lactam antibiotics) and some affect protein synthesis (macrolides, Chloramphenicol, Lincospectin, tetracyclines, aminoglycosides). Others compromise DNA and RNA synthesis (fluorquinolones, ansamycines) and some disturb the metabolism of e.g. folic acid (Trimethoprim).

The intention of a trial with these different groups of antibiotics was to evaluate possible interactions they may have with a combination of secondary plant compounds. Four ESBL producing *E. coli* field isolates from poultry flocks were experimentally assessed as well as a β -lactamase positive and a β -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 β -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](#).

Using egg immunoglobulins to enhance piglet survival



The number of healthy piglets weaned is the most important factor for the calculation of profit in piglet production.

Losses in the farrowing unit normally occur during the first seven days of life as piglets are born with very little protection in the form of immunity. The intake of immunoglobulins from colostrum is therefore of vital importance. Besides cleanliness and special feeding, piglets can be additionally supported by two strategies that mimic the effect of colostrum:

- a direct one, meaning the feeding of immunoglobulins (IgY from eggs) to piglets that would support the immune system in the gut or
- an indirect one, meaning a supply of IgY to the sow to keep the pathogenic pressure in the farrowing unit as low as possible.

Piglets are born with no immune protection and very low energy reserves

It is well known that piglets are physiologically immature at birth. Their energy reserves are very low with only 1 - 2% body fat comprising mainly of structural and subcutaneous fat. Therefore, in the first hours of life they rely on the glucose supply from glycogen from the liver as their main energy source. However, this will only cover their needs for a few hours.

Due to the construction of the sow's placenta, a transfer of immunoglobulins (antibodies) within the uterus is not possible. This means that piglets are born with practically no immune protection and depend on the immediate intake of immunoglobulins from colostrum. The immunoglobulins can be absorbed in the [gastrointestinal tract](#) and immediately transferred into the bloodstream - but also only for a short time. The absorption ability of the piglets starts to decrease soon after birth and ends after 24 to 36 hours.

Strategy 1: Making the farrowing unit as safe as possible

The piglets' environment should be warm to prevent hypoglycaemia. Piglets looking for heat close to the sow can also get crushed. Since the temperature needs of the sow and piglets are different, a piglet nest with a special heat lamp is recommended. Furthermore, the farrowing unit should be clean. Due to their low immune status, piglets are susceptible to common pathogens such as *E. coli*, *Clostridium perfringens*, and rotavirus that can all lead to diarrhoea.

Most pathogens can be traced to those found in the sow's faeces. To keep this amount as low as possible, different measures can be taken:

- A vaccination increases the immune defences of the sow. The antibodies fight against the pathogens so that less "functioning" pathogens are excreted.

- Feeding of probiotics increases the number of good bacteria like Lactobacilli and Bifidobacteria competing with the pathogens for binding sites and nutrients.
- Administration of [egg immunoglobulins](#), which bind to the pathogens within the gastrointestinal tract and make them harmless. These pathogen-immunoglobulin-complexes can be ingested by the piglets without any danger.

Strategy 2: [Supporting the piglets with immunoglobulins](#)

The aim here is to strengthen the local [immunity in the gastrointestinal](#) tract by increasing the amount of immunoglobulins (Ig). As already mentioned, the intake of sow colostrum is of vital importance. With the vaccination of the sow, the content of antibodies in the colostrum can even be enhanced.

An additional measure would be to orally supply the piglets with egg immunoglobulins (IgY). Both classes of immunoglobulins (IgG from mammals, and IgY from birds) can bind to pathogens in the gut, preventing them from binding to the intestinal wall and reducing the incidence of diarrhoea. The difference is in the degree of effectiveness and specificity.

Conclusion

To maximize the number of piglets weaned, it is necessary to support their immune system during the first days of life. Besides good hygiene management, the administration of [egg antibodies to the sow](#) will also help reduce the amount of shed pathogens keeping the pathogenic pressure low. The application of egg antibodies directly to the piglets supports their immune system by binding the pathogens in the gut, minimizing the risk of diarrhoea.

A powerful alternative to antibiotics for Aquaculture



Global aquaculture has grown dramatically and shrimp cultivation areas, in particular, have expanded. Unfortunately, the shrimp industry, in particular, faces major problems with bacterial diseases. One of the most important diseases in shrimp is vibriosis. Mortality rates of up to 100 % are possible, so economic losses can be devastating.

Characteristics of vibriosis

Vibriosis generally occurs in all life stages, but mainly in hatcheries. Vibrios are found as normal flora in the hepatopancreas of the healthy crustacean. They can turn from tolerated to pathogenic, if environmental conditions are compromised: e.g. over / underfeeding, overcrowding or decreased levels of oxygen.

The animals can be infected orally, through wounds in the exoskeleton or pores, the gills or the midgut. There are different expressions of the disease depending on which parts of the animal are affected (e.g. appendage and cuticular vibriosis).

Negative effects on the ecology

As antibiotics for shrimps are applied orally together with the feed, not all of them reach their target. An estimated 15-40 % are not ingested due to feeding falling to the bottom. A fraction of the ingested antibiotics is also not absorbed in the body and is excreted. All of these antibiotics stay in the water or sink to the bottom. The number of antibiotics that remain in the water or sediment varies from 1 % (chloramphenicol) up to 90 % (Oxytetracycline).

It is estimated that 70-90 % of antibiotics used in the therapy of farmed organisms end up in the environment and sediment and lead to the development of antibiotic resistance.

Secondary Plant Compounds (SPCs) - a good tool to reduce the use of antibiotics?

SPCs and their components are able to slow down or prevent the growth of molds, viruses, and bacteria. They impair them by acting at different parts/mechanisms of the cells (e.g. cell membrane, transport systems, cell contents, flagella development, quorum sensing...). The best-explained mode of action is one of thymol and carvacrol extracted from thyme and oregano. These substances are able to penetrate the bacterial membrane and disrupt its integrity causing loss of ions or energy equivalents.

Several trials conducted show the high efficacy of secondary plant compounds in aquaculture.

1. Scientific Trial (Kasetsart University, Thailand)

Design

a) 4 groups (6 replicates each) of White Leg Shrimp (*L. vannamei*) were housed in 100 L aquaria with 10 animals each.

Control: Standard feed, no additive

AB-Group: Standard feed + 10 ppm Enrofloxacin

Activo Group 1: Standard feed + 100 g Activo/t of feed

Activo Group 2: Standard feed + 200 g Activo/t of feed

Evaluation of mortality and specific growth

a) End of the feeding trial: stressing of the shrimp (high water temperature, 33°C for 1 hour), then challenge with *Vibrio parahaemolyticus* ($7,6 \times 10^6$ cfu/ml) by subcutaneous injection.

Evaluation of mortality

a) Survival rates in the AB-Group (93,3 %) and in the Activo Group 2 (90,0 %) were similar. The specific growth rate of the AB-Group (2,32 %/day) and the Activo Group 2 (2,22 %/day) were higher than the control (1,94 %/day). The Activo Group 1 (2,18 %/day) ranged performance-wise between the control and AB-Group.

b) After the challenge, mortality in the control group (43,3%) was approximately twice as high as in the AB-Group (20 %) and in the two Activo groups (both 23,3%).

2. Field Trial (Shrimp farm Ecuador)

Design

Two ponds with 80.000 shrimps/ha

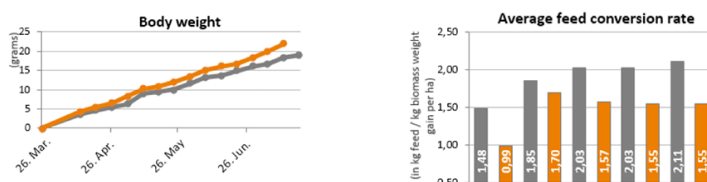
Control (3 ha): standard feed

Activo Aqua Group (5 ha): standard feed + 2 kg Activo Aqua (Activo upgraded by immune system stimulating- mannan-oligosaccharides) /t of feed on top

Evaluation of average shrimp weight at regular intervals

Results

Activo Aqua Group showed a consistently better development of body weight compared to the control, resulting in a shorter cultivation period until harvesting (112 compared to 123) and therefore a higher turnover of animals. Feed conversion in the Activo Aqua group was better in every growth stage.



Both trials present secondary plant compounds as a good alternative to antibiotic growth promoters. In the case of disease, they decrease mortality. Under standard conditions, the improved development shortens time to harvest and increases the turnover. The improved feed conversion lowers feeding costs.

Using milk thistle to reduce liver damage from mycotoxins



Mycotoxins not only reduce animal performance, but they also cause significant liver damage.

The seeds of the herb plant milk thistle contain a mixture of flavonolignans known as [silymarin](#) and can help in [reducing liver damage](#) when animals get in contact with mycotoxin contaminated feed.

Mycotoxins are a constant problem in cereals causing economic losses to the global animal industry. Mycotoxins are produced by filamentous fungi varying widely in their chemical and biological characteristics and effects on animals. Among the various mycotoxins, aflatoxins, and more specifically aflatoxin B1, is one of the most problematic because it affects maize, one of the major staple ingredients in animal diets worldwide. Of course, in nature, mycotoxins mostly occur in combinations, but even with singly contaminated ingredients, the nature of animal feeds leads to the concurrent presence of multiple mycotoxins, coming from the different ingredients. The separation of mycotoxins in polar and non-polar, however, simplifies their management. For example, aflatoxins (polar) are easily addressed by the inclusion of an adsorbent (like bentonite, for example). The same ingredient adsorbs not only aflatoxins, but also other mycotoxins, like zearalenone, ochratoxin A, and T-2 toxin, albeit at reduced efficiency.

Products limited to work in gut

Certainly, anti-mycotoxin agents are effective only while the feed is being

digested, that is, while the feed remains in the lumen of the [gastrointestinal tract](#). Anti-mycotoxin agents are not absorbed by the animal, whereas non-adsorbed mycotoxins are; leading to the need for further detoxification within the organism. Parts of mycotoxins might enter the organism despite the use of an anti-mycotoxin agent in feed due to the fact that no product is 100% effective, not all mycotoxins are affected similarly by a single product, non-polar mycotoxins might not be inactivated if only a polar agent is used, and vice versa and lastly, high contamination might render the normal dosage inadequate. This is often seen as being the most common cause, In other words, part of mycotoxins in the feed can still enter the animal. The exact effects on animal health and performance will depend, of course, on the initial contamination levels in the feed and on the constitution of the liver.

Mycotoxins and liver damage

Even short-term exposure to mycotoxins suffices to cause significant liver damage and loss of performance. In a study (Meissonnier, 2007), pigs were given 385, 867, or 1807 µg aflatoxin B1/kg feed for four weeks. Pigs receiving the highest level of aflatoxin developed clear signs of aflatoxicosis: hepatic dysfunction and decrease in weight gain. Also, the pigs exposed to the lower levels of mycotoxins showed clear signs of impaired metabolism and biotransformation. Additionally, mycotoxins and particularly aflatoxins inhibit the major hepatic biotransformation enzymes. This has significant consequences in veterinary medication applications as animals become unable to clear medications from their system – and of course, other toxins.

Read [Using milk thistle to reduce liver damage from mycotoxins](#) the full article
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