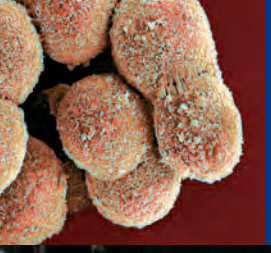


WHITE PAPER

Mycotoxins at low levels cause the greatest economic chronic losses in milk production & more diseases

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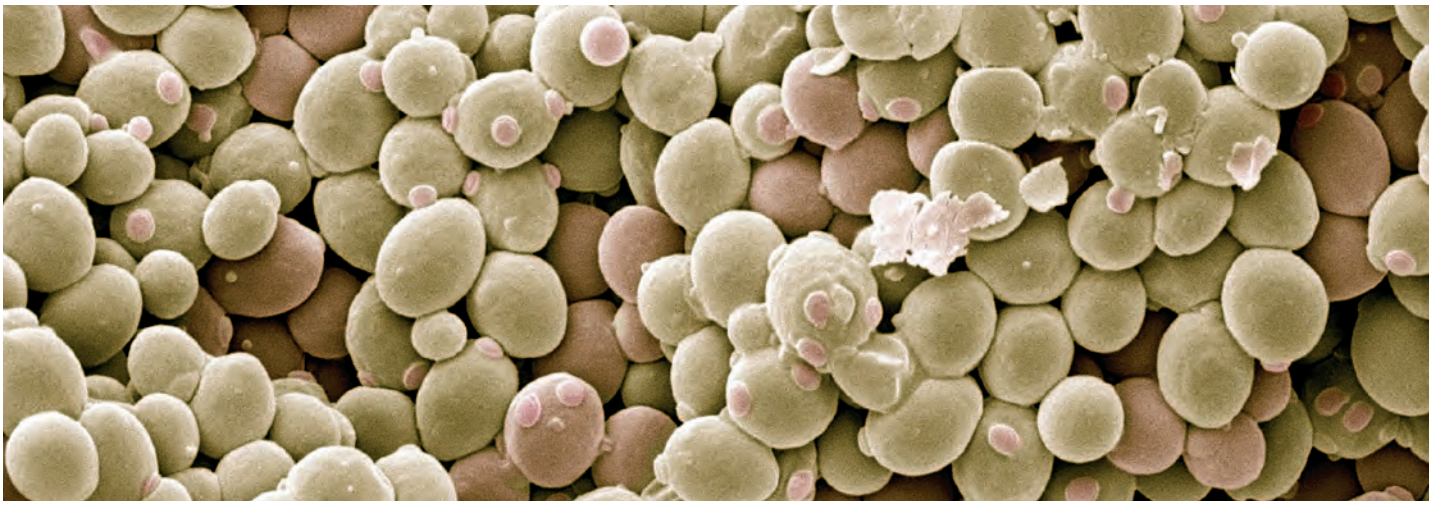
CONTENT



In today's environment, the presence of mycotoxins is an inherent risk.

The chances are very high that multiple mycotoxigenic molds including *Aspergillus, Fusarium, Penecillium* and related toxins are crept into silos and present in corn silage at harvest and after ensiling (recent survey highlighted that 99% of grain samples collected contained mycotoxins and over 85% multiple mycotoxins).

Because ruminants consume forages, byproduct feeds and wet feeds, they are exposed to a broader range of mycotoxins at concentrations that are perhaps higher than are found in dry grain mixtures.

Under normal conditions, a multiple-toxin contamination is likely.

Multiple toxins can have a synergistic effect, increasing the negative impact on animal's performance and health. Toxins from mold and fungus combined with bacterial toxins increase the negative health issues significantly.

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Escent® S

While the rumen microorganisms can do something to degrade a certain degree of toxins, rumen metabolites of such toxins may be equaly or more toxic.

It should always be considered that **mycotoxins will adversely impact rumen environment and activity** even before having an effect on the animals themselves. Decreases in ruminal motility, on DM, ADF and starch digestion and on microbial growth are some of the impacts seen in animals fed mycotoxin contaminated diets, directly impacting production and indirectly initiating other metabolic disorders.

However, a more likely scenario is to find mycotoxins at lower levels interacting with other stressors to cause more subtle symptoms leading to subclinical losses in performance, increases in incidence of disease and reduced reproductive performance. **To the dairy producer, these subclinical losses are of greater economic importance than losses** from acute effects, but even more difficult to diagnose.

Mycotoxins affect dairy cows

- Below normal milk production
- Inconsistent dry matter intake
- Reducing feed consumption
- Altering rumen fermentation
- Increasing incidence of opportunistic disease
- Causing cellular death
- Suppressing immunity
- Impacting Liver function
- Altering reproduction
- Irritating tissues
- Decreasing milk component production

Mode of Action

1 **Preventing Oxidative stress:** with selected protective antioxidants providing cellular support against the damaging effects of free radicals on intestinal micro-flora, tissues and cells.

- Mycotoxins are among the stress factors that have a negative effect on pro & antioxidant balance in the body and especially in the cell. This may lead to a situation whereby the cow is no longer able to quickly neutralise free radicals, leading to **oxidative stress**. An animal 's oxidative balance is one of the many factors that can limit milk production. Dealing with oxidative stress requires more energy from the animal that could otherwise be used for milk production, growth, longevity, fertility and overall animal productivity.

MALONDIALDEHYDE LEVELS IN BLOOD

Animal group	Experiment time, Days	malondialdehyde, µmoll/l
1 (Toxins)	Beginning	1,65±0,16
	10	3,07±0,21***
	20	7,32±0,22***
	30	8,69±0,19***
2 (Toxins) + Escent®	Beginning	1,74±0,18
	10	2,88±0,19**
	20	5,64±0,16***
	30	6,92±0,18***

* - p <0,05 ** p <0,01 *** p < 0,001

2 **Reducing immune suppression & strengthening of animal natural immune response:** with the use of selected plant extracts and specific, biological response modifyers.

- Chronic mycotoxins exposure increase susceptibility to infectious diseases weakening immune response.
  Most mycotoxins are immune suppressive. They are affecting the immune status of the cow partly because immune cells have membranes high in poly unsaturated fatty acids (PUFA) which are very susceptible to oxidation leading to destruction and tissue damage. Aflatoxins reduce antibody production, fumonisins reduce macrophage activation, ochratoxins inhibit all aspect of immunity, Don and Zearalenone increase the susceptibility to bacterial infections.

Parameters	Animal group			
	1 Contaminated	2 Contaminated + Escent®	3 Clean feed	4 Clean feed + Escent®
Antibodies titer	1:10,2	1:19,3	1:22,8	1:20,4
Protection level, %	60	85,7	90	90

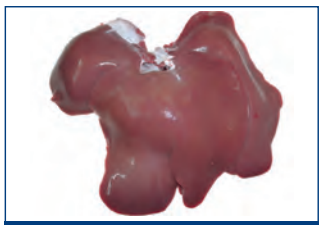
Response to vaccination

3 Liver & Kidney support with selected plant extracts proven to support these organs function in case of toxic stressors (toxin blockade at membrane level, protein synthesis enhancement, anti-fibriotic activity, anti- inflammatory effect...)

- It is the **cow's liver** that is affected when toxins contamination occurs and it needs to convert the toxins into something benign that can be excreted. Hepatic bio-conversions of mycotoxins will need to take place – risking liver overload and additional Reactive Oxygen Species – to change the polarity. Here liver may not be able to detoxify all those components.

Contaminated liver

Clay-colored liver unevenly colored (toxic dystrophy) noticeable spikes, flabby consistency.



Contaminated feed + Escent®

Homogeneously red colored, sharp edge liver

4 Biotransformation and binding of Fusarium producing toxins modifying such toxic molecules into less harmful or polar components.

5 Binding& Adsorption/Captation of water soluble (polar) toxins and reducing their bioavailability. Both high adsorbent mineral clays and yeast extracts rich in gluco-mannans are used to adsorb mycotoxins efficiently, selectively and quickly, reducing the bio-availability for the organism.

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Mycotoxins inactivator evaluations and study. The assessment of the effectiveness of Escent® S on dairy cow fed artificially contaminated feed

Date & Location: Quarter 3 2012, Federal Government Institution – Federal Center for Toxicological, Radiation and Biological Safety – Russia **Species:** Dairy cows

Evaluation:

- 3 treatments of 10 cows each for a 15 day period
- Artificial contamination:
  – 250 ppb of Zearalenone
  – 200 ppb of T2-Toxin
- **Escent® S** dosed at 30 g/h/d

The test performed at the Federal Center for Toxicology is very challenging as we put to test ESCENT® against the most difficult toxins in lactating dairy cows; DON, T-2 and Zearalenone in synergism.

T-2 and DON belong to the trichotecenes group having broad spectrum harmful effects on various body organs, systems and cell structure starting from gastrointestinal issues like leaky gut, vomiting, dermo-necrotic caustic ending, cell membranes peroxidation, impact on immunity, glucose, protein and mineral metabolism.

Based on the low milk production, level of glucose and LDG in the blood, this herd was most probably suffering from ketosis from the beginning of the experiment prior to the addition of artificial mycotoxin contamination.

Blood serum:

Parameter	Group						Physiological norm
	1(Control)		2 (Toxins)		3(Toxins + ESCENT)		
	Before the experiment	At the end of the experiment	Before the experiment	At the end of the experiment	Before the experiment	At the end of the experiment	
Whole protein, g/l	86,0±1,4	86,8±0,7	85,0±2,2	76,0±1,6**	84,0±1,4	83,6±1,2	83-86
amylopsin, U/l	55,8±0,4	52,6±0,5	50,1±0,6	58,6±0,7**	58,0±1,4	52,4±0,9*	до 60
Alkaline phosphatase, U/l	86,0±2,0	85,0±1,3	87,0±1,6	70,0±1,5***	81,0±0,6	80,0±0,6	до 100
ALT, U/l	54,0±1,3	51,3±0,7	48,8±0,5	53,0±0,7	48,8±0,4	49,5±1,0	до 55
AST, U/l	84,0±2,5	83,1±2,8	88,5±0,4	91,7±1,7	83,0±1,6	71,0±0,8***	70-100
GGT, U/l	8,5±0,2	8,5±0,2	8,2±0,3	8,5±0,4	8,4±0,1	8,5±0,1	7-10
LDG, U/l	860,0±9,3	814,5±4,3	872,0±2,7	622,0±2,9***	694,0±6,6	761,0±6,6***	до 1000

T-2 toxin is known to inhibit protein synthesis. Whole protein measurements (albumin produced by the liver) and globulin (made by the liver and the immune system), can indicate liver and immune system status. Animals in the contaminated group indicate compromised liver and immune systems as their whole protein blood levels decreased significantly from 85.0 g/l down to 76.0 g/l. Animals fed ESCENT® whole protein blood levels remain constant at 84.0 g/l. This clearly indicates a detrimental effect mainly from T2-toxin and a protective effect provided by ESCENT®.

Alkaline phosphatase (ALP) is produced by the muscles, liver and bone. Generally speaking abnormal levels of ALP in blood most often indicate a problem with liver and/or bones. However, it can also indicate malnutrition. The drop in ALT indicates a possible failure of the liver.

Looking at AST, an enzyme produced by the liver also indicates that in the presence of toxins contamination levels have gone up to 91.7 U/l indicating a higher amount to be found in the blood rather than in the liver where it belongs and needs to function. The ESCENT® treatment kept the level down to 71.0 U/l suggesting a better functioning liver.

Results:

No clinical signs of intoxication were detected. Feed and water consumption were the same. The obtained results evidenced the expressed **negative effect of mycotoxins on dairy cows liver function**. Using the **Escent® S** allows to decrease the toxins effect by its removal from the gastro-enteric system.

Milk Production:

Experiment day	Group		
	Control	Toxins	Toxins + ESCENT S
Average value during the whole experiment	20,06	19,14	19,8
Production rate during the experiment	301,0	287,1	297,8
		-4,6% versus control	+3,7% versus Toxins fed group

Escent® S provides an increase in milk production thanks to its ability to reduce mycotoxins adsorption and to inhibit the endogenous toxic aggregates.

This research confirmed that using the **Escent® S** mycotoxin inactivator in lactating cow diets contaminated with mycotoxins offers a protective effect against toxins and promotes animal welfare.

Dairy Evaluation

Date & Location: Q1-Q2 2015 , Milano, Italy **Species:** Holstein Dairy cows

Experiment:

- University of Milano
- 200 cows – TMR fed
- 2 treatments
- 30 days before calving till 150 after calving
- Natural contamination (Afla , Zea, DON)
- Avg Milk production 36 kg/c (+/- 11 000 kg /c/y)
- **Escent® S** at 35 g/h/d

Results:

- Red blood cells (KRL) : 10% improvement
- NEFA in the liver : Trend to have less with Escent®
- Lower body loss
- ALP reading better
- Ovarian cyst reduced
- Cows in second lactation : +3,8kg milk*
- Cows in third lactation : + 5,2 kg milk*

** Raw milk*

Finding the dietary solution to toxins, stress and immunity in dairy cows.

Dr Rüdiger Kratz – Technical Services – Ruminant. INNOVAD SA/NV (Belgium)

The entire dairy industry, including consulting nutritionists, veterinarians and producers, all strive to keep their herd in good health knowing that healthy cows will be able to cope better with stress, especially with potentially contaminated feedstuffs.

Stress resulting in oxidative stress can negatively impact the dairy cow. Molds and mycotoxins, endotoxins, hidden toxins in the feed, extra heat, pathogens challenges, environmental issues, changes in diets, transition period and calving all compromize the cow's immunity system and its ability to deal with possible diseases, causing immune suppression. As a result, higher somatic cell counts, lower milk yield, poorer reproduction performance, mastitis and metritis, are observed.

Molds are omnipresent. Their main task in nature is to decompose organic matter. More than 400 mycotoxins have been identified but about 20-30 are frequently detected with highly sensitive analytical methods (LC/MS-MS) in feed and food in higher concentrations. The most critical mycotoxins for ruminants are deoxynivalenol (DON), zearalenone formed by Fusarium spp. and aflatoxin B by Aspergillus.

Fumonisins, ochratoxin A, ergot alkaloids as well as silage-associated roquefortin C and mycophenolic acid can also be detected.

The formation of mycotoxins undergoes significant regional and seasonal variation and among other things depends on the nutrient supply, water content in the substrate and in the surrounding air, temperature and pH. The optimum conditions for mold growth and toxin formation do not necessarily need to coincide.

Molds and mycotoxins in feed cause chronic, 'subacute' problems in dairy cattle that show up with signs of higher disease incidence, reduced fertility or sub-optimal milk production.

This is mediated by the following modes of action:

- Reduced intake or feed refusal.
- Altered microbial growth in the rumen.
- Reduced nutrient absorption and impaired metabolism.
- Altered endocrine and exocrine systems.
- Suppressed immune function.

Experience from research and practice indicates that individual actions are not sufficient.
The best way to eliminate such risks related to the concurrent presence of toxic contaminants along with all other stresses inherent to the cows' production challenges seems to lay in a combination of actions – the cow's metabolic support emphasizing maintenance and balancing oxidative stress management, the essential organ (liver mainly) aid, the stimulation of rumen function and immune response, along with the reduction of mycotoxins adsorption and toxins toxicity through their bio-transformation.

Balancing oxidative stress

In biological organisms, such as the dairy cow, the antioxidant system and pro-oxidative substances (reactive oxygen species (ROS) are finely regulated at cellular level. Many studies have shown oxidative stress as a fundamental factor of unwanted immune and inflammatory responses.

Dairy cows, especially in the phase from gestation to lactation, are susceptible to a variety of diseases. ROS affect the regulation of gene expression, and the antimicrobial activity of the macrophages. Elevated levels of ROS damage nucleic acids, proteins and lipids, affecting important physiological functions. Food spoilage and mycotoxins are considered oxidative stress triggers. It is not yet completely clear whether this is done by direct stimulation of the formation of ROS or indirectly by weakening the antioxidant system. Presumably, both paths are taken.

In most cases, the levels of natural antioxidants are reduced due to lipid peroxidation caused by mycotoxins.

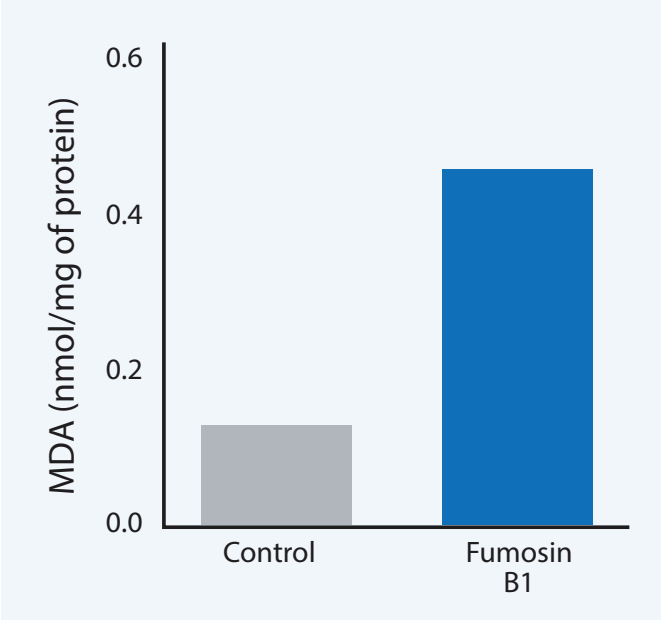

Figure 1. Oxidative activity of Fumonisin B1 on kidney cells (Abado-Becognee et al 1998).

Fumonisin B1 was found to be a strong inducer of malondialdehyde ***(marker of oxidative stress, see Fig. 1)***

The antioxidant system of the mammalian cell is complex and consists of proteins, enzymes, vitamins and pro-vitamins, which are found in the cytosol, mitochondria or cell membrane.

Special secondary plant metabolites such as the polyphenols can stabilize the existing system. Polyphenols are a complex group of substances, which can be divided into phenolic acids and flavonoids and being subdivided much further. They play an important role in

building the cell walls that protect the plant from harmful influences such as UV light and pathogens and are involved in the repair of cellular damage.

The absorption of the polyphenols occurs mainly in the small intestine *(Fig. 2)*. They may be chemically modified, bound on albumin to become water soluble and reaching the liver via the portal vein. In the liver, other molecular changes take place, such as hydroxylation, decarboxylation and conjugation, having the polyphenols become hydrophilic and excreted via the kidneys in the urine.

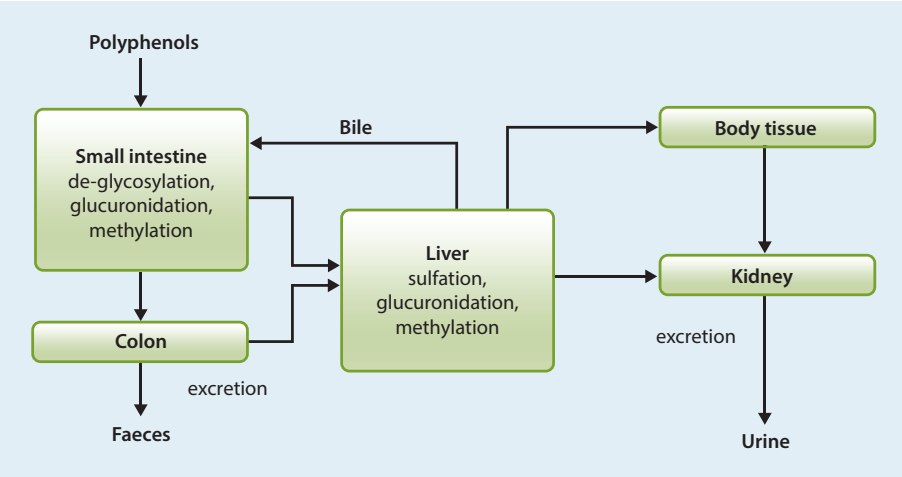

Fig. 2. Metabolism of polyphenols.

Thus, the main sites of action for polyphenols are the intestinal mucosa, liver, and kidneys. The structural variability of polyphenols is also reflected in their effect. For example proanthocyans are very poorly absorbed and their effect remains limited on the intestinal mucosal area.

Flavanones and isoflavones show the best bioavailability and can exert their antioxidant potential in blood, liver and kidneys. However, the concentrations fall quickly after stopping supply, so that constant feeding is necessary. The antioxidant potential of polyphenols can be measured in relation to vitamin E in Trolox equivalent antioxidant capacity (TEAC), showing a broad variation of <0.1 to >5.0 mM TEAC per mM polyphenol. Therefore the usage of polyphenols presupposes their effectiveness in terms of absorption and antioxidant capacity.

Supporting liver function

Crucial organs, such as the liver, are stressed and damaged or malfunctioning due to the presence of mycotoxins (aflatoxin and fumonisin) after absorption, while immune function is compromized by most of the other mentioned toxins.

The liver has a very high metabolic activity that makes it extra vulnerable for oxidative stress by aggressive molecules. In addition, the liver of dairy cows during early lactation is exposed to specific extra stresses. Low concentrations of glucose and insulin in the blood and increased influx of free fatty acids lead to fat deposition in the liver. Molds and mycotoxins can exacerbate this further by reducing feed intake. Some herbal ingredients have been proven to protect the liver. Experience with various parts of plants or extracts are supported by trials with cell cultures (in vitro model), animal studies (in vivo model) and clinical trials in humans.

Rosmary is well known for its strengthening effect on liver functions. Production and flow of bile are stimulated, so that the digestion is improved. The glucuronidation of unwanted molecules is increased, leading to accelerated elimination via urine and diminishing their potential disease impact. Artichoke leaves are a liver detoxifying and regenerating agent. It is mainly used to treat liver dyspepsia and disease. Main active components are cynarine and other bitter substances resulting in the regulation of lipid digestion.

Stimulating rumen function

It should always be considered that mycotoxins will adversely impact the rumen environment and activity even before having an effect on the animals themselves. Decreases in ruminal motility, on dry matter intake, acid detergent fibre (ADF), starch digestion and microbial growth are some of the

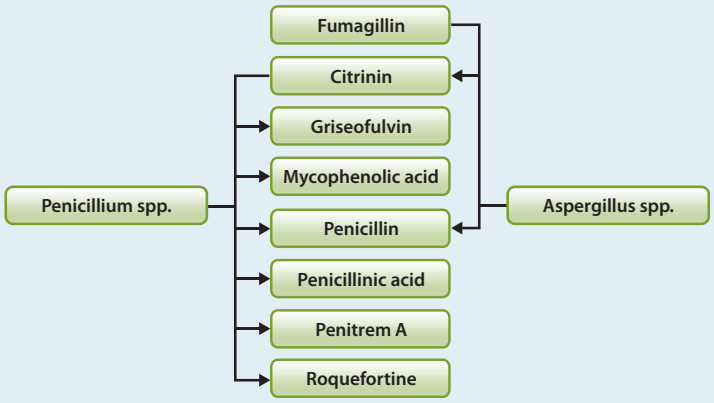

Fig. 3. Antibiotics produced by Penicillium and Aspergillus spp.

issues seen in animals fed mycotoxin contaminated diets, directly impacting production and indirectly initiating other metabolic disorders.

Additionally, toxins like aflatoxin and deoxynivalenol reduce feed intake and by consequence further suppression of nutrient supply. In dairy cattle, T2-toxin has been associated with intestinal hemorrhages, bloody faeces, gastrointestinal lesions and enteritis, finally disrupting the digestive process in the lower part of the digestive tract.

Molds also produce antibiotics to defend themselves against other mold and bacteria. ***Fig. 3*** shows some antibiotics produced by penicillium and aspergillus spp. present in silages. These antibiotic activities will suppress bacterial production in the rumen and lead to decreased feed conversion as well as 'normal' toxic effects of mycotoxins.

Fermentation extracts can maintain rumen functioning and performance even in the presence of mycotoxins. They supply micronutrients like B vitamins, branched chain fatty acids and oligopeptides to a variety of bacteria and protozoa and stimulate their growth and efficiency acting therefore as prebiotics.

Cellulolytic bacteria are especially supported and may be increased in numbers by about 50%, bacteria +15%. As a result, the digestibility of organic matter, ADF and hemicellulose are improved. The production of short-chain fatty acids can be increased, indicating higher energy supply from feed fibre.

Supporting immune function

Mycotoxins appear to have a significant immunotoxic potential, depending on the degree of exposure. Gliotoxin produced by A. flavus acts as an immunosuppressive, being antibacterial and improving apoptosis. These effects can be enhanced further by T-2 toxin, as it inhibits phagocytosis of A. fumigatus conidia by macrophages. Direct effects of T-2 toxin are seen in lower concentrations of plasma immunoglobulin, and protein. Cows in phases of stress as in early lactation or due to high temperatures are particularly susceptible to mycotoxins because their immune system is already overtaxed.
The interactions between the immune system and nutritional status or requirements are well documented. The requirement of the immune system is highly dependent on the immune response and applied conditions. The system is less stressed when vital organs such as the liver are fully functional.

The rumen has great potential to eliminate toxins, if the microflora is well balanced and very active. In addition, the immune system can be activated directly. B-glucans, as extracted and concentrated yeast cell walls, can activate leukocytes and cytokines. Cytokines are peptides and some regulate growth and differentiation of cells, others are mediators of immunological reactions. The stabilization of the immune system results in fewer cases of mastitis, and lower concentration of somatic cell count.

Conclusion

At the beginning of lactation, during high mobilization of body reserves and with high feed bypass through the rumen, the cow can barely cope with an additional burden like mycotoxin contamination. A multi-functional approach should be used to maintain and to stabilize the health of the cow naturally. Innovad's Escent® can keep the liver and kidney healthy, as well as keeping the rumen highly productive, resulting in more milk. ■

WHITE PAPER

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Mycotoxins Risk Assessment

"….The biggest challenge in the mitigation of toxins contamination is the ability to properly detect the risk we are confronted with…"

Prof Trevor Smith , University of Guelph, ON, Canada

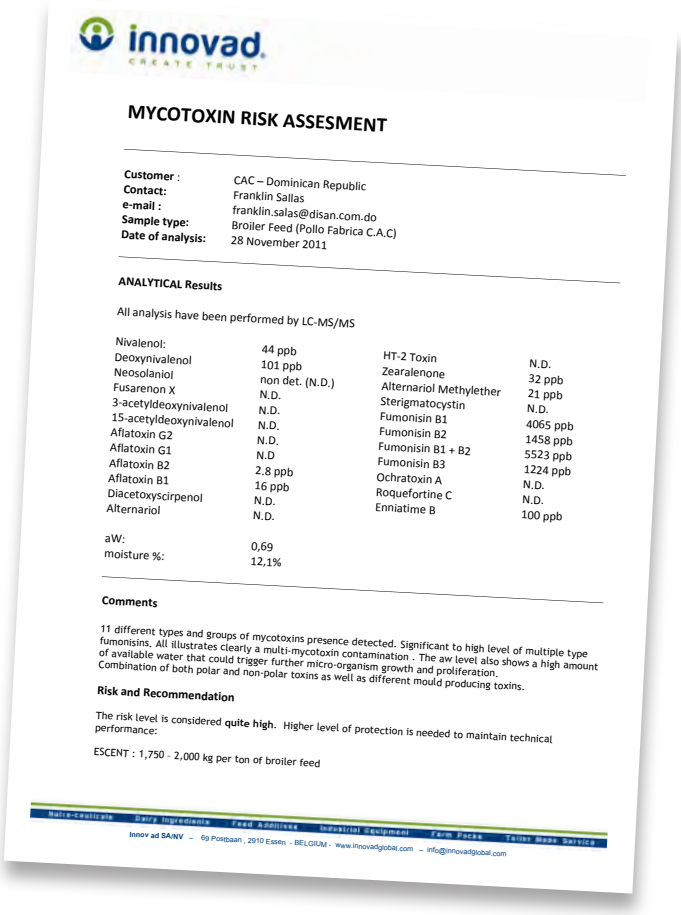
INNOVAD® has developed a specific service to help assess the potential contamination risk and performance losses related to the presence of toxins. Making use of analytical techniques and an own database, INNOVAD® offers practical solutions based on accurate recommendations.

Samples are collected and sent for analysis following LC-MS/MS procedure.

From **6 to 22 mycotoxins** can be analyzed , along with 3 other key indicators (moisture, pH, aW). Basic information about sample feed history, raw materials usage and animal specie & age feed application are compiled along with analytical results. All data are put in INNOVAD® data base and processed by INNOVAD® technical experts for risk calculation.

With this tool, INNOVAD® can

- Perform mycotoxin analytical assays
- Analyze the results, compare with data base
- Evaluate the potential contamination risk based on analytical results, historical information, specie/age and health status of the animals and environment pressure
- Provide a Mycotoxin Risk Assessment Report & Diagnosis per sample
- Make an accurate recommendation on how to use **Escent®**
- Keep data base per client
- Provide trend and analysis on risk evolution



MYCOTOXIN RISK ASSESMENT

Customer : CAC – Dominican Republic
Contact: Franklin Sallas
e-mail : franklin.salas@disan.com.do
Sample type: Broiler Feed (Pollo Fabrica C.A.C)
Date of analysis: 28 November 2011

ANALYTICAL Results

All analysis have been performed by LC-MS/MS

Nivalenol:	44 ppb	HT-2 Toxin	N.D.
Deoxynivalenol	101 ppb	Zearalenone	32 ppb
Neosolaniol	non det. (N.D.)	Alternariol Methylether	21 ppb
Fusarenon X	N.D.	Sterigmatocystin	N.D.
3-acetyldeoxynivalenol	N.D.	Fumonisin B1	4065 ppb
15-acetyldeoxynivalenol	N.D.	Fumonisin B2	1458 ppb
Aflatoxin G2	N.D.	Fumonisin B1 + B2	5523 ppb
Aflatoxin G1	N.D	Fumonisin B3	1224 ppb
Aflatoxin B2	2.8 ppb	Ochratoxin A	N.D.
Aflatoxin B1	16 ppb	Roquefortine C	N.D.
Diacetoxyscirpenol	N.D.	Enniatime B	100 ppb
Alternariol	N.D.		
aW:	0,69		
moisture %:	12,1%		

Comments

11 different types and groups of mycotoxins presence detected. Significant to high level of multiple type fumonisins. All illustrates clearly a multi-mycotoxin contamination . The aw level also shows a high amount of available water that could trigger further micro-organism growth and proliferation. Combination of both polar and non-polar toxins as well as different mould producing toxins.

Risk and Recommendation

The risk level is considered **quite high.** Higher level of protection is needed to maintain technical performance:

ESCENT : 1,750 - 2,000 kg per ton of broiler feed

Innov ad SA/NV – 69 Postbaan , 2910 Essen - BELGIUM - www.innovadglobal.com – info@innovadglobal.com

Escent® S

When to use

- When low feed quality is suspected
- When intakes are reduced
- During stressful periods
- In the suspected presence of toxins

Direction to use

Escent® S: 10-40 g/h/d depending on stress & risk

- Milking herds : 15-30 g/h/d
- Transition & early lactation: 20 g -40 g/h/d
- Heifers: 10-15 g/h/d to ensure consistent feed intake

Escent® Dairy Pack is also available for use on TMR: 40-100 g/h/d

Innov ad sa/nv
69 postbaan, 2910 Essen – Belgium
Tel. +32(0) 3 667 16 55
Fax +32(0) 3 670 05 05
www.innovad-global.com
info@innovad-global.com