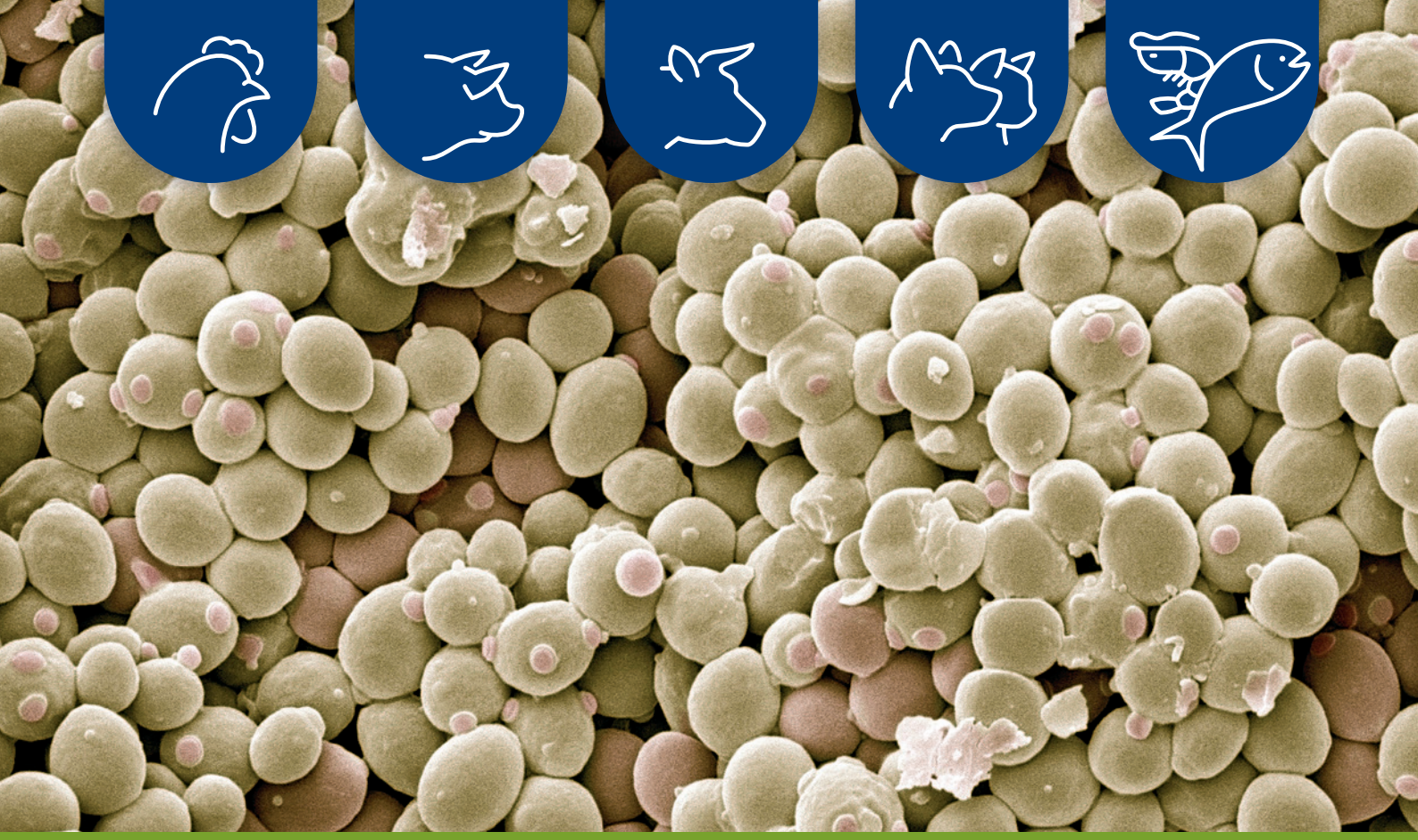


Improve productivity
Control toxins and stress

Escent® S Platform

A SCIENTIFICALLY PROVEN TECHNOLOGY & A UNIQUE SERVICE

Mycotoxin & Stress Control

innovad®
CREATE TRUST

What is the Escent® S Platform ?

1 A product technology with 5 modes of actions

1. Supports liver and kidney
2. Prevents oxidative stress
3. Restores natural immunity
4. Boosts natural detoxifying processes
5. Binds polar (water soluble) toxins

Pages 4-7

2 A NEW diagnostic service assessing:

a) the risk of mycotoxins in feed AND

b) the TRUE IMPACT OF MYCOTOXINS in animals using ONLY one drop of blood on FTA cards, with LC-MS/MS

BLOOD and impact Analysis
+ FEED Risk assessment

Pages 10-11

3 A liquid revitalizing intervention for drinking water application at critical periods

Page 12

4 A Science based Platform

1. Peer reviewed publications
2. Product trials database (both experimental and field trials in multi-species)
3. Novel research on Animal Mycotoxin-Biomarkers of Exposure


toxins

Assessment of Dried Blood Spots for Multi-Mycotoxin Biomarker Analysis in Pigs and Broiler Chickens

Pages 8-9

The real Multi-Parametric Challenge

Stress:
How to alleviate stress in animals potentially confronted with toxic contaminants

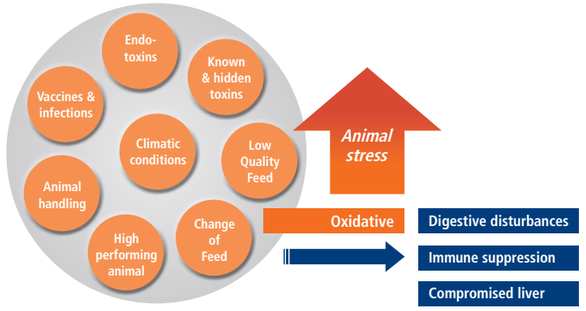


Fig. 1. The complex interactions between stress, inflammation, and the health of the animal.

Aiming to resolve solely the issues raised by the presence of mycotoxins in feed is an inadequate approach

Stress factors such as animal handling, sudden environmental changes, heat, diet composition changes, poor feed quality, vaccinations and disease challenges are affecting severely animal health and productivity.

Farming animals possess limited natural resistance and immunity against such stressors which often lead to oxidative stress, where the animal is no longer capable of detoxifying adequately the reactive oxygen species formed at cell level.

The negative impact of such stress factors is worsened and amplified in the presence of toxic contaminants.

- Toxic contaminants decrease the function of key organs such as the liver and kidneys.
- Mycotoxins, as part of toxic contaminants, have a significant negative impact on the defense mechanism, immune system and reproductive systems of animals.
- For example, Fumonisins and DON increase the paracellular gut permeability and predispose to the development of necrotic enteritis in broilers whereas, DON in pigs inhibits vaccination efficiency of PRRSV live vaccines.

Only multi-functional preventive approaches guarantee high performance in today's challenging production conditions

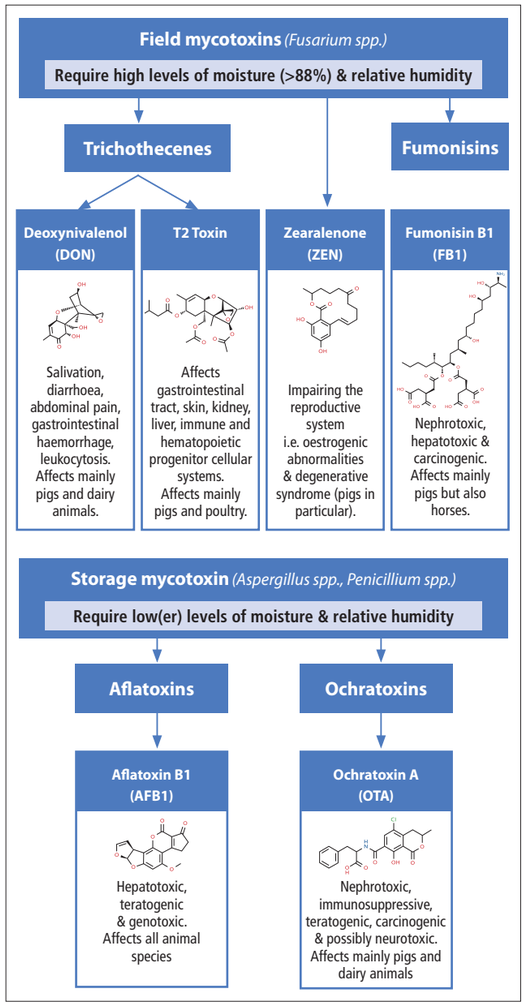


Fig. 2. The main groups of mycotoxins found in animal feed, the microorganisms responsible for their formation, source of formation, the main mycotoxin(s) per group and their main effects and animal species susceptibility.

The synergistic negative effect of multiple mycotoxins

Chronic exposure even to low levels of multiple mycotoxins weakens the immune response and increases susceptibility to infectious diseases due to a combined negative effect.

The combined negative effect of multiple-mycotoxins is **exacerbated** when **combined** with other **stress factors**.

The animal health and productivity are then compromised to a much greater extent with **significant economic losses** for the producer.

The Escent® S Technology

Escent® S

A multi-functional preventive approach to counteract **stress** in the context of toxic contaminants and guarantee **high performance** in today's challenging production conditions

The primary aim is to transform non-soluble toxic chemicals into water-soluble chemicals so that they can be excreted via bile or urine.

Escent® S expands beyond traditional binding approaches of clays as these are practically ineffective against non-polar toxins. The **Escent® S** Technology supports the natural detoxification (both Phase I and Phase II - see **Fig. 3**) processes within the liver.

Escent® S
expands beyond traditional binding approaches which are practically **ineffective** against **non-polar** toxins

Liver detoxification remains a key target

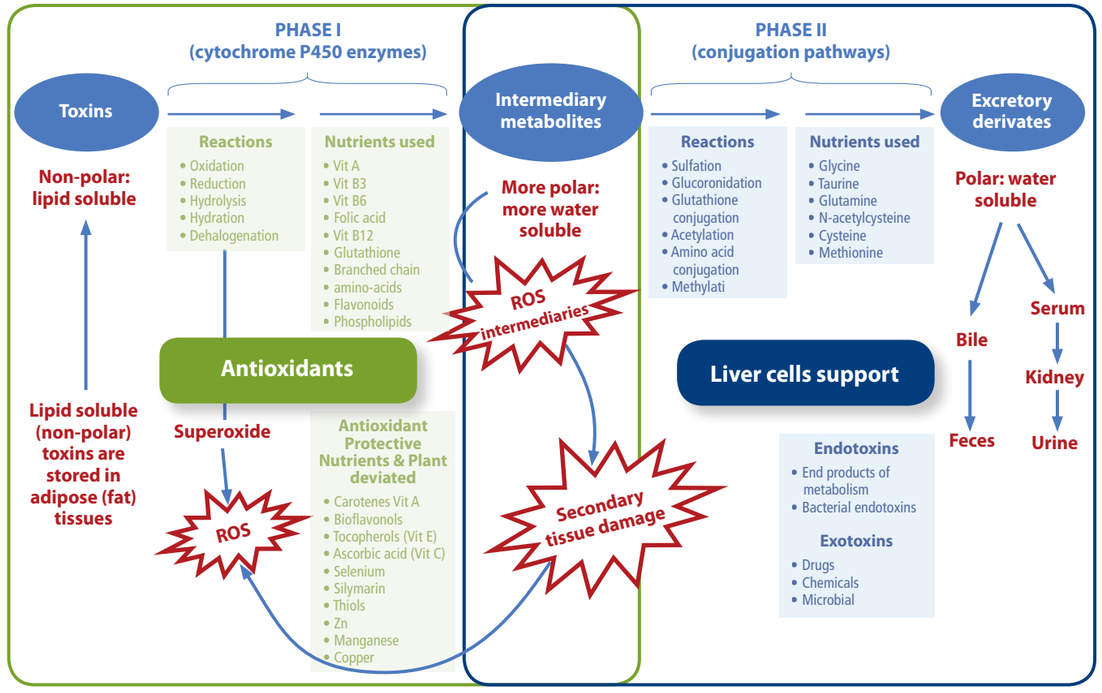
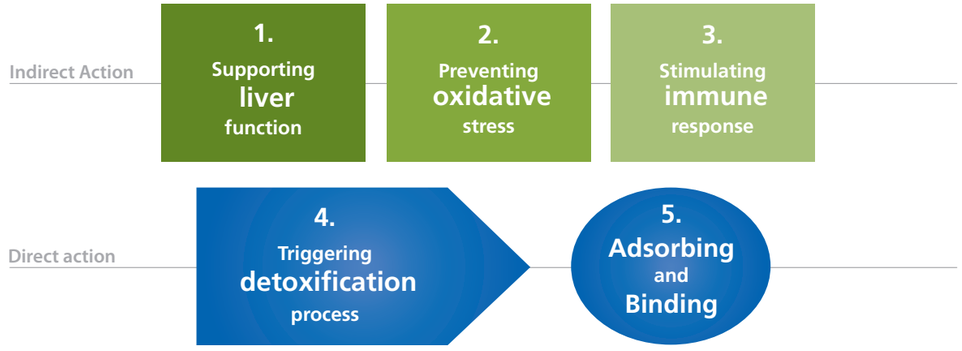


Fig. 3 Phase I and phase II detoxification processes occurring in the liver (adapted from Liska DJ, 1998)

Escent S® - Scientifically proven modes of action:



1

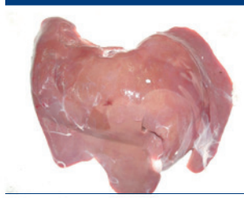
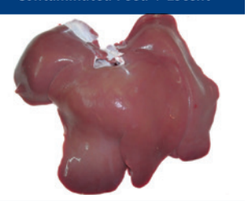
Liver and kidney support

With selected plant extracts proven to support the function of these organs when confronted with toxic stressors (e.g. toxin blockade at membrane level, protein synthesis enhancement, antibiotic activity, anti-inflammatory effect etc.).

Enzymatic activity of liver tissue in the afore mentioned mycotoxicosis study in piglets revealed that several biomarkers including **bilirubin**, alanine transaminase **(ALT)**, aspartate aminotransferase **(AST)** and alkaline phosphatase (**ALP**) were all statistically elevated in the Control group (data not shown). Moreover, **histology** examination of **key organs** at the end of the trial (30DPI) revealed that **Escent® S** improved significantly all signs of mycotoxicosis in the **Escent® S** group when compared to the Control group.

Calculation of the liver coefficient (liver weight/body weight) demonstrated the positive impact of **Escent® S** versus the Control group (**Escent® S**; 0.029±0.001 and Control group 0.034±0.001 - p<0.001).

Representative images of post-mortem liver examinations.

Contaminated liver	Contaminated Feed + Escent®
	
Clay-coloured liver unevenly coloured (toxic dystrophy) noticeable spikes, flabby consistency.	Homogeneously red coloured, sharp edge liver

2

Preventing Oxidative stress

With selected protective antioxidants providing cellular support against the damaging effects of free radicals on the intestinal microflora, cells and tissues. Oxidative stress correlates to elevated levels of lipid peroxidation which is linked with a universal non-specific mechanism of several pathologies within the organism. Elevated oxidative stress consumes significant amounts of energy from the animal that could otherwise be used for growth, longevity, fertility and overall animal productivity.

A clinical trial on pig mycotoxicosis was performed in weaned piglets in the Federal Centre for Toxicological, Radiation and Biological Safety in Russia. The piglets were challenged with several mycotoxins; ZEN: 50 ppb; DON: 1,000 ppb; T-2: 70 ppb, without or with **Escent® S** (2kg/Tn feed). Comparison of oxidative stress (malondialdehyde) in blood revealed statistically significant protection in the group treated with **Escent® S** throughout the study (**Table 1**).

Table 1. Comparison of malondialdehyde levels in blood of weaned piglets challenged with several mycotoxins; ZEN: 50 ppb; DON: 1,000 ppb; T-2: 70 ppb, without or with **Escent® S** (2kg/Tn feed).

Malondialdehyde levels in blood

EXPERIMENTAL TIME (DAYS)	MDA (µmol/l)	
	Control (Toxins)	(Toxins) + Escent®
Beginning	1.65±0.16	1.74±0.18
10	3.07±0.21*	2.88±0.19*
20	7.32±0.22**	5.64±0.16**
30	8.69±0.19**	6.92±0.18**

* p <0,01 ** p < 0,001

3

Counteracting **immune suppression** and strengthening the animal's natural immune responses

With the selected plant extracts and specific immunological response modifiers. Chronic exposure even to low levels of multiple mycotoxins weakens the immune response and increases susceptibility to infectious diseases due to a combined negative effect.

Pigs

The humoral response to Classical Swine Fever vaccination in the pig mycotoxicosis study revealed that the **Escent® S** group exhibited nearly 100% increased **antibody titres** at the end of the trial (30 DPI) when compared to the Control group (1:19.3 and, 1:10.2, respectively – **Table 2**).

Table 2. Response of antibody titres against Classical Swine Fever vaccination.

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

Antibody response to Classical Swine Fever vaccination

Chickens

In an experimental research trial in Lithuania, two hundred (200) male broiler chickens ROSS 308 were followed until 42d. The feed included naturally Fusarium-contaminated cereals containing DON. A statistically significant reduction of the Newcastle Disease vaccination efficacy was observed in the control group but this negative impact was removed completely by dietary inclusion of **Escent® S** at 1,5 kg/T feed (2.16±1.28 vs. 5.39±0.78, respectively $p < 0.01$; **Table 3**).

Table 3. Impact of **Escent® S** on the humoral immune response of male broilers (ROSS 308) assessed by the measurement of the titres to Newcastle Disease vaccination. The feed was naturally contaminated with deoxynivalenol (DON).

Note: Different alphabetical superscripts denote statistically significant differences, $p<0.05$.

Treatment	NEWCASTLE DISEASE TITRES	
	(U/litre)	St. Dev
Control feed	5.96 [a]	1.07
Contaminated feed	2.16 [b]	1.28
Contaminated feed + Escent® S	5.39 [a]	0.78
Contaminated feed	6.54 [a]	1.35

4

Enhancing natural **detoxification** processes

By modifying toxic molecules into less harmful or polar components. **Escent® S** supports both Phase I and Phase II detoxification processes in the liver whilst maintaining the balance between the two phases (see **Fig. 3**, p. 4).

Unique Biomonitoring Research performed at the University of Ghent, Belgium proved the positive effect of Escent® S on animal systemic detoxification

Escent® S demonstrated systemic reduction of several mycotoxins in biological fluids in chickens and pigs, while the animals were contaminated with blends of mycotoxins, with some being dosed several times higher than the EFSA guidelines

For full details see pg.8-9.

5

Binding and **adsorption**/captation

of water soluble (polar) toxins and reducing their bioavailability. **Both** highly adsorbent mineral **clays** and **yeast extracts** rich in gluco-mannans are utilised for the efficient absorption of polar mycotoxins, reducing thus, their toxic bio-availability within the organism.

Extensive *in vitro* research at the University of Missouri, USA showed that **Escent® S** exhibits significant binding capacity against a wide range of key mycotoxins at two different pHs **(Fig. 4)**. However, extrapolation of *in vitro* findings to *in vivo* systems remains highly speculative and true evaluation can be performed only in live animals.

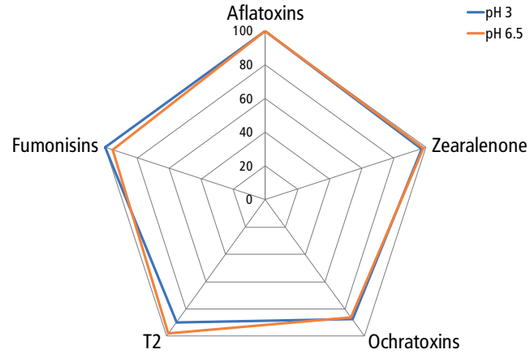


Fig. 4 *In vitro* testing of the binding capacity of **Escent® S** against several mycotoxins at the University of Missouri, USA.

Biomarkers of exposure (biomonitoring analysis): A new tool for the evaluation of mycotoxin detoxifiers

Escent® S is the unique mycotoxin detoxifier and stress control Technology that diminishes effects associated with mycotoxins.

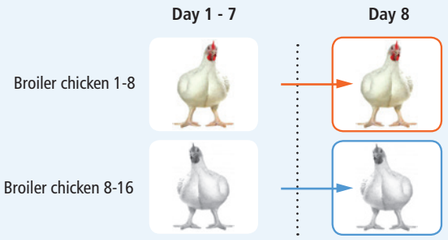
Performing *in vivo* toxicokinetic trials and not *in vitro* tests is a more accurate approach to test the efficacy of such technologies. Concentrations of mycotoxins and Phase I & Phase II metabolites in animal matrices are detected and the difference in concentration with and without detoxifier is determined by frequent sampling with the use of high accuracy analytical equipment (LC-MS/MS and HRMS). This difference is a true measure of the efficacy or impact.

Innovad® sponsored a four-year PhD research at the University of Ghent, Belgium on **Escent® S** which has yielded three scientific publications (papers) in the highly ranked journal of 'Toxins'.

The **first paper** involved the necessary Method Development and the validation of the appropriate 'Mycotoxin biomarkers for exposure' in several biological fluids (blood plasma, urine, faeces and excreta) in pigs and chickens. In other words, someone needs to 'prove' which mycotoxins and (Phase I & Phase II) metabolites should be targeted and bio-monitored for a specific animal species and a specific biological sample, as this relates directly to their unique metabolism for each animal species and each route of dissemination *in vivo*.

The **second paper** proved the *in vivo* efficacy of **Escent® S**.

POULTRY TRIAL - Experimental design

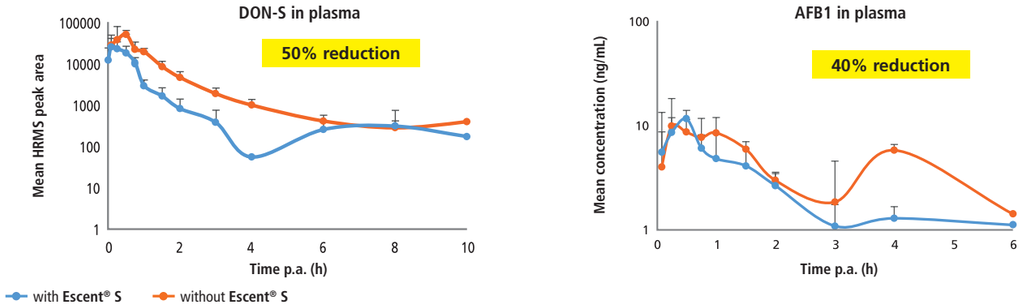


16 broiler chickens were acclimatized during one week.

After one week 8 chickens received mycotoxins and the 8 other chickens received mycotoxins plus **Escent® S** (2.4 kg/ton feed). The selected mycotoxins were aflatoxin B1 (AFB1; 20 ppm in feed), ochratoxin (OTA; 2.5 ppm in feed) and deoxynivalenol (DON; 5 ppm in feed).

Escent® S and the mycotoxins were administered using an intra-crop bolus. Plasma was collected.

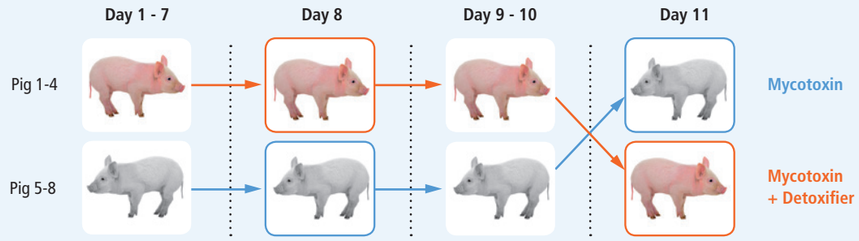
Results of the trial in plasma



Escent® S is the only Technology in the market to-date that has shown: A positive effect on chicken systemic detoxification. Namely, systemic reduction of mycotoxins in blood.

50% reduction of DON, 40% reduction of AFB1, while the animals were contaminated with a blend of three mycotoxins (DON, OTA and AFB1) with the levels of OTA being 25x higher and AFB1 1000x higher than the EFSA guidelines. The findings are of great importance taking into account the direct toxicity of **DON (and other mycotoxins)** and their predisposing role in **Necrotic Enteritis in chickens**.

PIG TRIAL - Experimental design



Age + number of animals: 6 weeks at start trial, 8 animals, cross over
Dose of detoxifier: Escent® S 0.1 g/kg BW or 1.0 kg/ton of feed
Dose of mycotoxins: Deoxynivalenol 36 µg/kg BW or 0.9ppm Zearalenone 3 mg/kg BW or 75ppm

- **Escent® S decreased numerically the systemic concentration of DON and ZEN** in plasma and urine of pigs **(Table 4)**.
- **Escent® S** demonstrated a positive effect on detoxification and lowered the impact of toxins on the pig metabolism despite **Escent® S** being **applied only at 1kg/Tn and NOT at 3kg/Tn (as initially intended)** due to human error.

  The findings are of great importance taking into account the direct toxicity of DON and ZEN in pigs, their predisposing role in a spectrum of pig **diseases** and their **negative impact on live PRSS vaccines**.

Table 4: Mean toxicokinetic parameters determined after single oral administration of DON (0.9 ppm) and ZEN (75 ppm) to pigs, either with **Escent® S** (n=8) or without (Control, n= 8).

BIOMARKER OF EXPOSURE	TREATMENT OR CONTROL	% DIFFERENCE OF THE AREA UNDER CURVE BETWEEN ESCENT® S AND CONTROL
DON-GlcA in plasma	Escent® S	-13%
DON-GlcA in plasma	Control	
ZEN-GlcA in plasma	Escent® S	-12%
ZEN-GlcA in plasma	Control	

Analytical Methodology: dried blood spots (DBS)

We then (**third paper**) transferred successfully the Analytical Methodology from (liquid) blood plasma to dried blood spots **(DBS)** with the **same analytical sensitivity** both for mycotoxins and phase I and phase II metabolites. The method validation results for pigs are shown in **Table 5**. Similar results were obtained for chickens.

Only a drop of blood is collected on a filter paper. The **methodology** has now been **transferred** to the unique **Myco-marker® Service (Patent Pending)** which enables for the first time with an easy way, the precise quantification of the **systemic (blood) levels of mycotoxins in animals** under real field conditions.

Table 5: Validation results for linearity shown as mean ± standard deviation of three curves across three different days of analysis (linear range, correlation coefficient (r) and goodness-of-fit coefficient (g) and limit of quantification (LOQ) of 23 mycotoxins of pig whole blood extracted from an 8 mm disk of dried blood spots.

ANALYTE	LOQ (ng•mL-1)	LINEARITY (n=3 different days)	
		r ± SD	g ±SD
ZEN	1.0	0.994 ± 0.002	12.2 ± 3.6
AZEL	1.0	0.996 ± 0.002	13.2 ± 4.0
AZAL	1.0	0.996 ± 0.001	16.4 ± 3.0
BZAL	0.5	0.995 ± 0.003	18.8 ± 1.8
BZEL	0.5	0.995 ± 0.003	16.2 ± 3.7
ZAN	1.0	0.998 ± 0.002	14.3 ± 3.9
TeA	1.0	0.996 ± 0.002	19.3 ± 0.4
AOH	2.0	0.998 ± 0.001	18.1 ± 1.5
AME	1.0	0.997 ± 0.002	16.5 ± 3.6
DON	1.0	0.992 ± 0.002	14.8 ± 3.0
DOM1	1.0	0.995 ± 0.001	14.9 ± 2.2
3/15ADON	0.5	0.994 ± 0.003	17.7 ± 1.4
T2	0.5	0.998 ± 0.001	15.6 ± 4.9
AFB1	1.0	0.993 ± 0.002	11.8 ± 2.5
AFM1	0.5	0.996 ± 0.003	13.4 ± 1.62
OTA	1.0	0.996 ± 0.001	14.3 ± 4.9
ENNA1	0.5	0.995 ± 0.002	15.3 ± 3.1
ENNA	0.5	0.997 ± 0.002	17.5 ± 1.5
ENNB	0.5	0.997 ± 0.001	11.8 ± 6.8
ENNB1	1	0.995 ± 0.003	10.4 ± 1.9
BEA	0.5	0.993 ± 0.001	14.5 ± 3.3
FB1	1.0	0.995 ± 0.002	16.5 ± 2.6
FB2	1.0	0.998 ± 0.001	11.3 ± 5.1

Measure the RISK and TRUE IMPACT of mycotoxins in animals

innovad
CREATE TRUST

PATENT PENDING

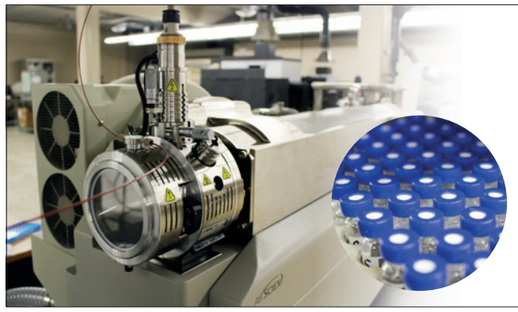
Myco-Marker®

Blood & Impact Analysis + Feed Risk Assessment

Myco-Marker® is a patent pending **DIAGNOSTIC TOOL** to evaluate possible exposure to mycotoxins and their impact on animal health.

Myco-Marker® allows detection and diagnosis of mycotoxin-mediated health and performance issues either in individual animals or in a population, helping to identify the causative mycotoxins and developing appropriate mitigating strategies.

1 Analytical **EXAMINATION OF RISK** in complex feed matrices and grains by monitoring 16 key mycotoxins in feedstuffs with high resolution LC-MS/MS technology.



Test Code	Result (dry basis)	Units	RL*	Risk Assessment*** Low / Moderate / High	Produced primarily by:
Deoxynivalenol (DON)	3,080	ppb	60		Fusarium/Gibberella
3-Acetyl-Deoxynivalenol	< 60	ppb	60		Fusarium/Gibberella
15-Acetyl-Deoxynivalenol	280	ppb	60		Fusarium/Gibberella
Total DON**	3,360	ppb	60		Fusarium/Gibberella
Fumonisins (B1, B2)	300	ppb	100		Fusarium/Gibberella
Ochratoxin A	< 3	ppb	3		Aspergillus/Penicillium
T-2	< 60	ppb	60		Fusarium/Gibberella
HT-2	< 60	ppb	60		Fusarium/Gibberella
Aflatoxins (B1, B2, G1, G2)	< 1	ppb	1		Aspergillus
Zearalenone	700	ppb	30		Fusarium/Gibberella
Diacetoxyscirpenol	< 60	ppb	60		Fusarium/Gibberella
Sterigmatocystin	< 30	ppb	30		Aspergillus

*Reporting Limit
**Total of Deoxynivalenol (DON), 3-Acetyl and 15-Acetyl forms
***Risk assessment is for informational purposes only. Effects of mycotoxins vary depending on age, environment, and additional stress.
External environmental factors have to be taken into account.
Additional stressors on farm need to be considered.

2 Analytical **DETECTION OF BIOMARKERS OF EXPOSURE IN BLOOD** from dried blood spots with HR LC-MS/MS analytical tools.*

Benefits:

- Easy sampling: only a drop of blood is required (~60µl)
- Reduced animal invasiveness
- Easier sample transport and storage
- No need for plasma separation
- No need for special procedures or tubes

** Assessment of Dried Blood Spots for Multi-Mycotoxin Biomarker Analysis in Pigs and Broiler Chickens, Toxins 2019, 11(9), 541.*

3 Interpretation of blood and feed analysis **RESULTS** and differential diagnosis support.

4 Appropriate product strategy and dosing **RECOMMENDATIONS**.

Escent® L
A liquid revitalizing intervention for drinking water application

Escent® S Platform also includes a liquid intervention, applicable via drinking water.

Drinking water offers a perfect medium for the application of **Escent® technology** helping:

- **to overcome periods of stress**
- **to recover faster from possible disorders**
- **to limit periods of reduced feed intake**

In case of emergency, or specific needs, when in feed supplementation is not feasible, such drinking water tool is ideal, economical and easy to apply.

Escent® L use:

Treatment can be started for all clinical or subclinical cases of mycotoxicosis as well as circumstances of stress, sever disease conditions, immune-suppression or whenever animal performance is reduced or health compromised.

Escent® L dosage:

Via drinking water : 0,5 - 1 ml/l during specific period of stress.

Consult our technical team for adequate recommendation.

Escent® S dosage:

Escent® S:

Monogastrics: 0.5 - 2.5 kg/ton
Ruminants: 15-40 g/h/day

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