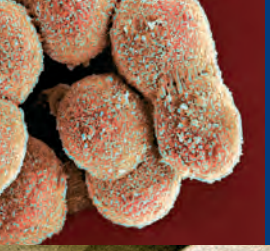


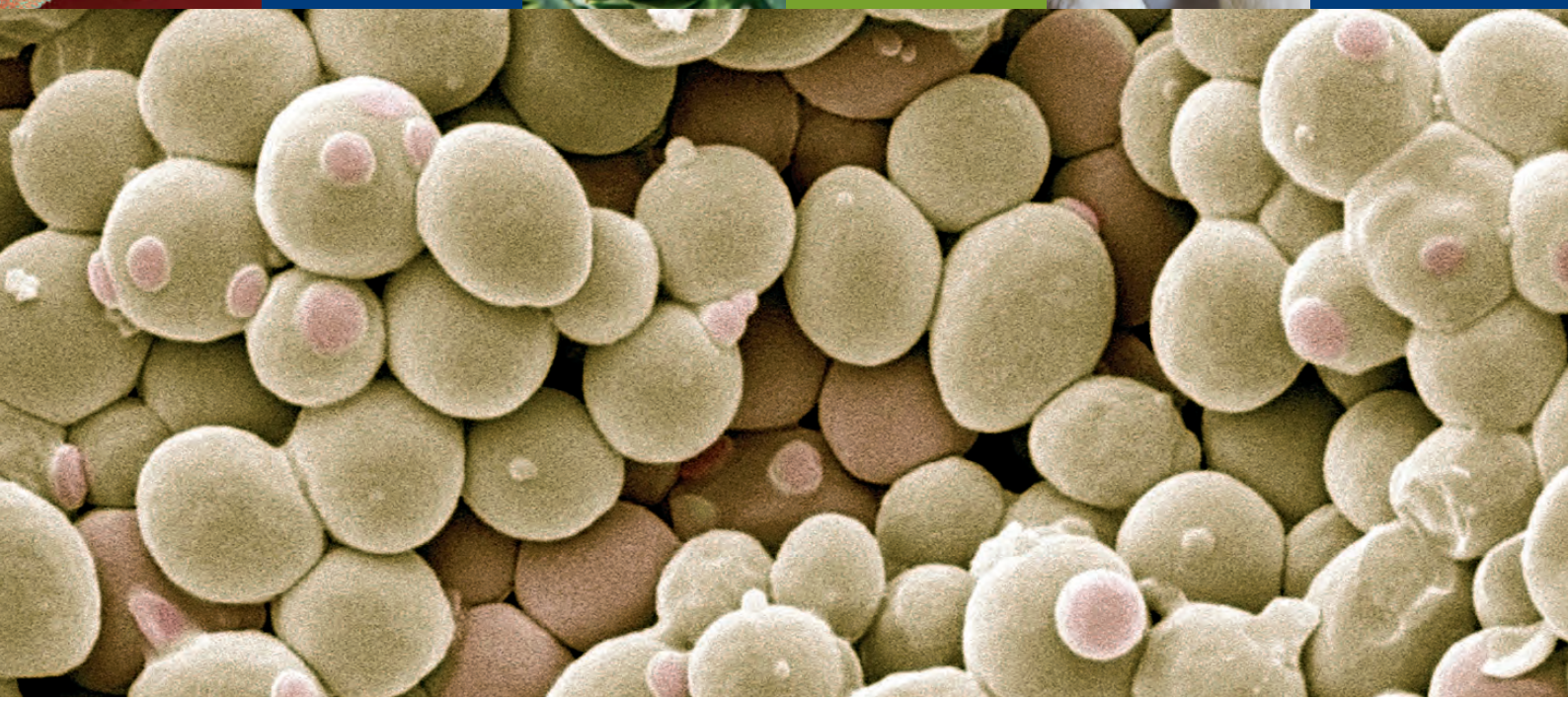

Escent®

Essential multi-task protection, support and prevention

(Escent® is not available in the USA and Canada)

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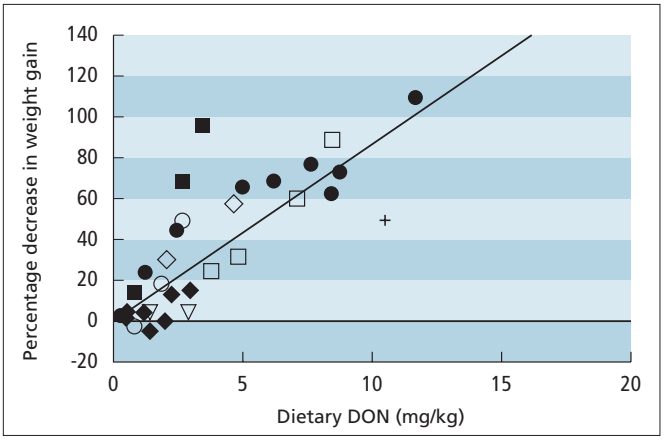
Toxins & Mycotoxins: The Issue

- Worldwide **more than 25% of the grain produced every year is contaminated** with mycotoxins. The increased global trading is enlarging the problem because the contaminated grains are blended with non-contaminated batches.

- **More than 400 types of mycotoxins have been identified**, which are diverse in their chemistry and their effects on animals.

- Feed contaminated with mycotoxins causes a serious range of problems including reductions in feed intake, growth performance *(see fig. 1)*, reproductive-, health and immunity problems *(see table 1)*. Symptoms are often non-specific and cost the agri-sector billions of dollars.

Fig 1: For every ppm increase in toxin concentration, the estimated reduction in growth rate in pigs is 16% for aflatoxin and 8% for DON (Dersjant-Li et al.,2003)



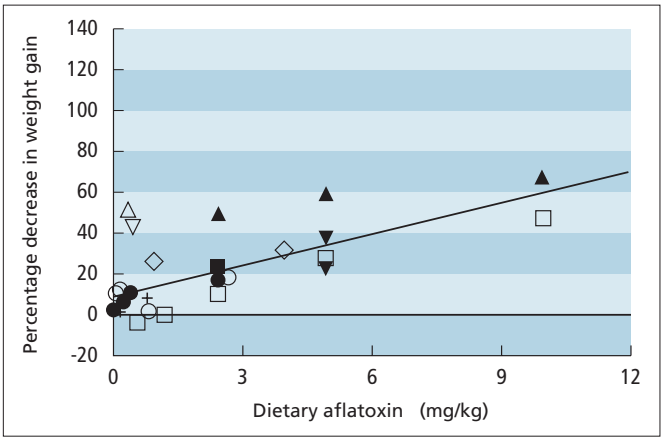


Table 1: Effect of Ochratoxin A on broilers' immunity and the pathogenicity of Salmonella. (Gupta et al., 2005)

TREATMENT	WEIGHT GAIN (G)	MORTALITY (%)	AST (IU/L)
Control 0	875a	0	50a
S. gallinarium	720b	6	55a
OTA	700b	0	68b
OTA + S. gallinarium	550c	15	73b



- Dietary mycotoxins can also end up in animal products destined for **human consumption**, such as milk, eggs and meat where they remain as stable and inert toxic molecules.

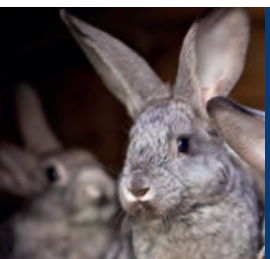
- Under normal conditions, a **multi-toxin contamination** is likely, which can have a synergistic effect, increasing the negative impact on the animal's performance and health. Also, the combination with bacterial toxins increases the health issues of mycotoxins significantly.

- Mycotoxins may also occur in conjugated form, either soluble (masked mycotoxins) or incorporated into/associated with/attached to macromolecules (bound mycotoxins).

- Due to the heterogeneity of contaminations, the expensive analysis and the relatively long analysis time, **individual sampling and analysis** is a critical factor and is often insufficient or too expensive to make a full risk evaluation. Therefore prevention should be a key element in an efficient approach.

Table 2: Only a handful mycotoxins out of the 400 mycotoxins known and their impact on farm animals' performance and health.

MYCOTOXIN	FUNGUS	MAIN EFFECTS	SPECIES SUSCEPTIBILITY
Aflatoxin (B1, B2, G1 and G2)	Aspergillus flavus; Aspergillus parasiticus	– Liver damage – Reduced feed intake – Immunosuppressor	All domestic animals and poultry
Ochratoxin A	Aspergillus ochraceus Pencillium viridacatum	– Kidney problems – Immunosuppressor – Dirty eggs with urine patches	Mainly pigs and dairy animals
Deoxynivalenol (DON)	Fusarium graminearum	– Vomiting, Feed refusal – Reduced milk production – Immunosuppresive	Mainly pigs and dairy animals
Zearalenone	Fusarium graminearum	– Decreased reproductive performance.	Mainly pigs and dairy animals
Fumonisin	Fusarium monliforme	– Pulmonary edema – Weakening of immune system	Mainly pigs and horses
T-2 toxin	Fusarium triicinctum	– Oral lesions, loss of appetite decreased egg production decreased hatchability	Mainly pigs and poultry



Escent®: Mode of Action

Escent®

Essential multi-task protection and support.

Each mycotoxin has different physical and chemical structures and feedstuffs are naturally contaminated with various toxins. Therefore, functional mycotoxin control requires various modes of action.

Escent® is a blend of different active ingredients that ensure a multifactorial approach to provide protection and support in 5 different ways:

Reduction of immunosuppression

A reduced status of the immune-system is alleviated by the use of Beta-glucans and well selected plant extracts.

Reduction of oxidative stress

The presence of mycotoxins plays an important role in lipid peroxidation at cell level. *(see fig. 2)*
Both synthetic and natural antioxidants avoid further action of free radicals towards intestinal micro-flora, tissues and cells, protecting the natural pathways of biotransformation of mycotoxins.

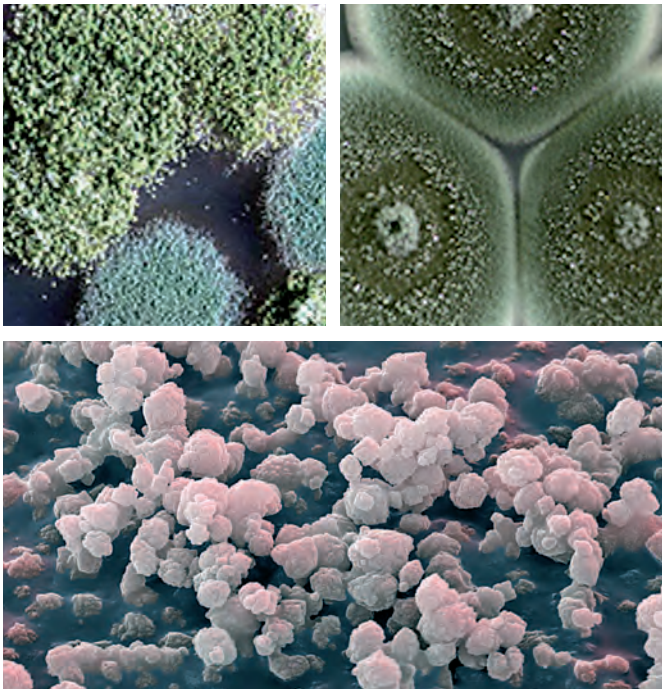
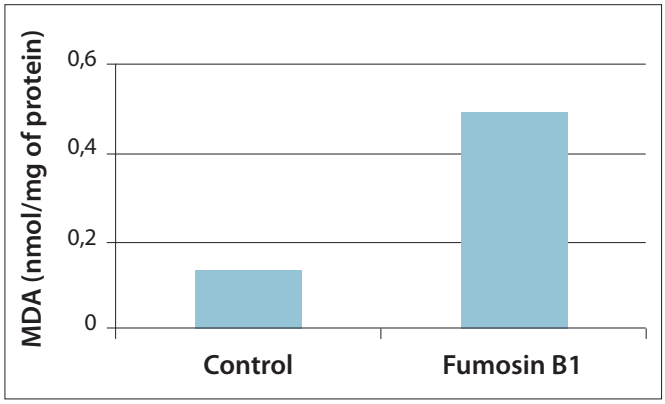


Fig. 2: Oxidative activity of Fumonisin B1 on kidney cells (Abado-Becognee K et al., 1998)

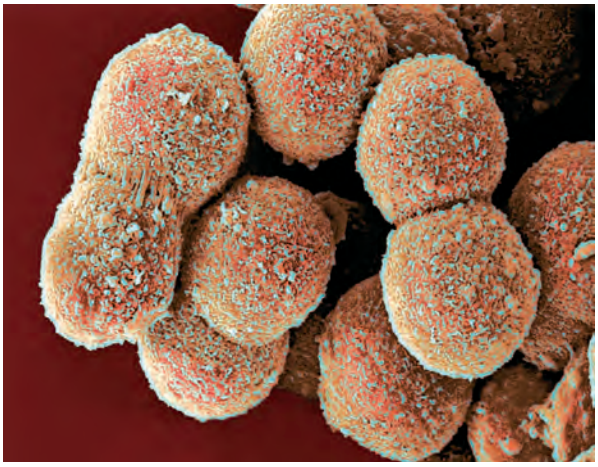






Organ support

The liver and kidneys are crucial organs in the detoxification and elimination of toxic principles in the blood. Several mycotoxins have a well-known impact on the functioning of these organs. **Escent®** contains various plant extracts, selected for their ability to maintain and restore organ function in case of toxic stressors.



Mycotoxin adsorption

Both highly adsorbent mineral clays and yeast extracts rich in gluco-mannans are used to adsorb mycotoxins. Their role is to adsorb mycotoxins efficiently, selectively and quickly, reducing the bio-availability for the organism.

Mould inhibition

In order to eliminate further mould development, and by consequence potential mycotoxin production, a selected mould inhibitor makes **Escent®** a well-balanced mixture between support, protection and prevention.



Essential multi-task protection, support and prevention

Escent® dry

in feed application

innovad®
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Trial Reviews



p. 10 - 13 ■ **AFLATOXINS**
– *in vitro*
– *in vivo*

p. 14 - 19 ■ **ZEARALENONE**
– *in vitro*
– *in vivo*

p. 20 - 29 ■ **DON**
– *in vitro*
– *in vivo*

p. 30 - 33 ■ **FUMONISINS**
– *in vitro*
– *in vivo*

p. 34 - 41 ■ **Others**
– *in vivo*

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AFLATOXINS

In Vitro Binding of AFLATOXINS

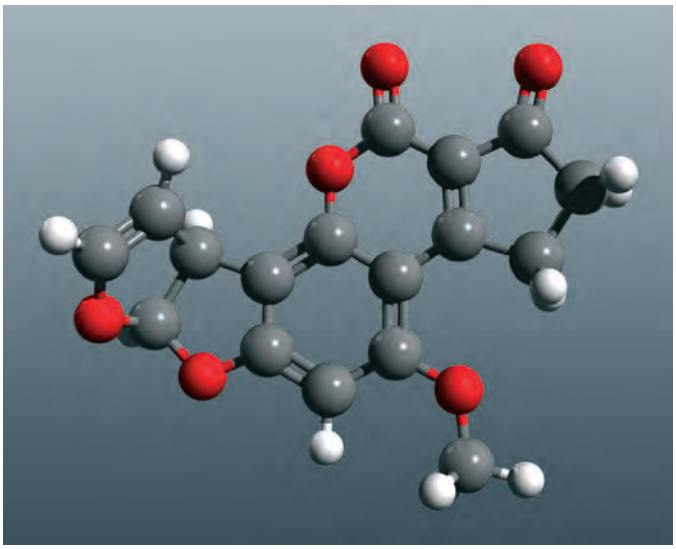
Date & Location: February 2011 - USA **Species:** *in vitro*

Introduction:

Mycotoxins are toxic fungal metabolites that are chemically diverse and can occur in a variety of grains, feed, food and beverage products.

Some of these compounds can cause serious human and animal health problems. Mycotoxins can enter the food and feed supply during production, processing, transportation or storage. Trilogy can test for more than 33 mycotoxins including Aflatoxin, Fumonisin, Ochratoxin and Vomitoxin.

Trilogy uses sophisticated chromatography methods including GC/ MS, GC and HPLC.



Methodology & protocol

- **Escent®** Inclusion Rate = 3.0 kg/ton
- 3,000 ppb toxin concentration
- Adsorption pH = 3.0
- Desorption pH = 6.5

Results : AFLATOXIN

% Adsorption :	99,6 99,7 99,7
% Adsorption Average	**99,7**
% Desorption	1,1 0,3 0,2
% Desorption Average	**0,5 %**
% Efficiency	**99,2 %**

Escent® is not available in the USA and Canada.

The effect of Escent® on the performance and liver health of broilers fed a diet contaminated with aflatoxins.

Date & Location: Sep 2010, Brazil **Species:** Broilers

Introduction:

Aflatoxins cause a variety of effects in poultry, including retarded growth rate, impaired feed conversion, increased liver weight, immunosuppression, negative effects on serum chemistry and hematological parameters, and histopathological lesions. Aflatoxins are very liposoluble compounds and are readily absorbed from the gastrointestinal tract into the bloodstream. Aflatoxins tend to infiltrate most of the soft tissues and fat deposits of the chicken. However, the majority of the accumulation occurs in organs such as the liver and kidneys (Lesson et al., 1995)

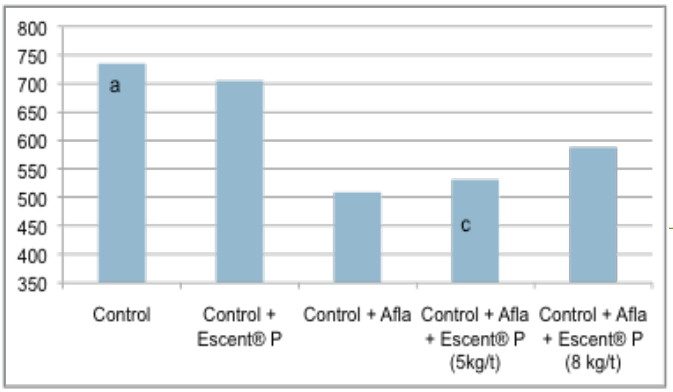
Protocol:

Protocol designed following the guidelines from the Brazilian Ministry of Agriculture for anti-mycotoxins product registration approval.

- 420 broilers (Cobb 0, 49g day old weight
- 7 treat * 6 reps * 10 birds
- 21 days – ad libitum
- 2.8 ppm Aflatoxins+**Escent®** at 5 & 8 kg/ton of feed

* Statistically significant

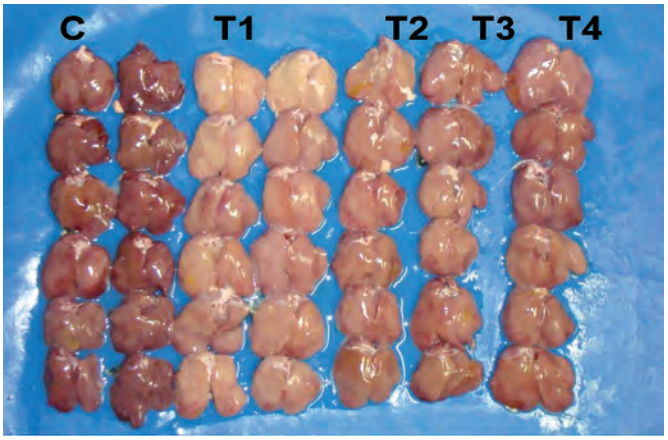
Results (Average weight gain at 21 days)



Escent® Trial data

The effect of **Escent®** on average weight (g at 21 days), relative liver weight (g/100g BW) and total plasma proteins (g/dL) of broilers fed a diet with extremely high contamination of **aflatoxin** (2.8 ppm) (Brazil, 2010)

CODE	TREATMENT	AVERAGE WEIGHT (G)	LIVER WEIGHT (G/100G BW)	TOTAL PLASMA PROTEINS (G/DL)
C	Control	734,94a	2.86c	3
T1	Control + 2.8 ppm Afla	508.76c	5.14a	1.45
T2	Control + 2.8 ppm Afla + 2.5 kg/ton **Escent®** P	533.07c	5a	2.01
T3	Control + 2.8 ppm Afla + 5 kg/ton **Escent®** P	531.14c	4.94a	2.16
T4	Control + 2.8 ppm Afla + 8 kg/ton **Escent®** P	587.69b	4.38b	2.23



C: non contaminated livers; T1: contaminated livers;
T2,3,4: livers from **Escent®**

Conclusions:

A clear negative impact of 2,8 ppm aflatoxins on:

- Live weight at 21 days (-30,8%)
- Feed intake at 21 days (-32,9%)
- Relative liver weight (+79,7%)
- Total Plasma Proteins (-51,7%)

The use of **Escent®** (8 kg/ton) to the contaminated diet has a positive effect on:

- Live weight at 21 days (+15,5%)
- Feed intake at 21 days (+18,3%)
- Relative liver weight **(-14,8%)**
- Liver coloration

The use of **Escent®** in the control diet did not have a significant impact on the technical performance.

Escent® is not available in the USA and Canada.

ZEARALENONE

In Vitro Binding of Zearalenone

Date & Location: USA (Mid West University) – March 2011 **Species:** *in vitro*

Introduction:

Experimental plan

Mycotoxins - Zearalenone, was purchased from Sigma Chemical Co. Primary stock solutions of each mycotoxin (1,000 ppm) were prepared in methanol. Mycotoxin test solutions for adsorption tests were prepared by adding methanol stock solutions to 0.1 M phosphate buffer adjusted to pH 3 or distilled water for the ergot alkaloids. Mycotoxin concentrations are based on the relative ease of analysis by HPLC and cost of mycotoxins rather than levels known to cause problems in livestock.

Binding studies – **Escent®** was tested for its ability to bind the mycotoxins (zearalenone) at pH 3 using the following general procedure. Duplicate aliquots of 0.1 M phosphate buffer (adjusted to pH 3) containing 2 ppm zearalenone, in solution (10 ml) were added to 15 ml screw cap Falcon polypropylene tubes to which 0.1 gram of each adsorbent had been added. In order to eliminate exogenous peaks, controls were prepared by adding 10 mL of 0.1 M phosphate buffer plus 100 mg adsorbent to test tubes. Test tubes were placed on a rotator shaker for 30 minutes at room temperature. The mycotoxin test solution and control was centrifuged at 13,000 rpm for 5 minutes and 2 ml of the aqueous supernatant removed for mycotoxin analysis. An aliquot of the original buffered mycotoxin test solution was used as the HPLC standard for the mycotoxin.

Analysis - HPLC analyses were performed on a Hitachi L-7100 pump with a Hitachi L-7200 autosampler, fluorescence detection with a Hitachi L-7480 fluorescence spectrophotometer and UV detection with a Hitachi L-7400 detector. Data were recorded and processed by a Hitachi D-7000 data acquisition package with ConcertChrom software on a microcomputer. The percentage of bound mycotoxins was calculated from the difference between the initial and final concentration of mycotoxin in the aqueous supernatant.

Methodology & protocol

- 3,000 ppb toxin concentration
- Adsorption pH = 3.0
- Desorption pH = 6.5

Results ZEARALENONE:

% Adsorption at pH 3.0 :	60,40%
% Adsorption at pH 6.0	**76.60 %**

Escent® is not available in the USA and Canada.

Scientific Experiment with Rabbit feeds contaminated naturally with ZEARALENONE

Date & Location:
October - November 2011, University Warmińsko-Mazurski in Olsztyn (Poland) with the Prof. Andrzej Gugołek.
Species: Rabbits

Treatments :

1. Control contaminated
2. Positive with **Escent®** 3kg /T - 26 rabbits per group

Mycotoxins contamination :

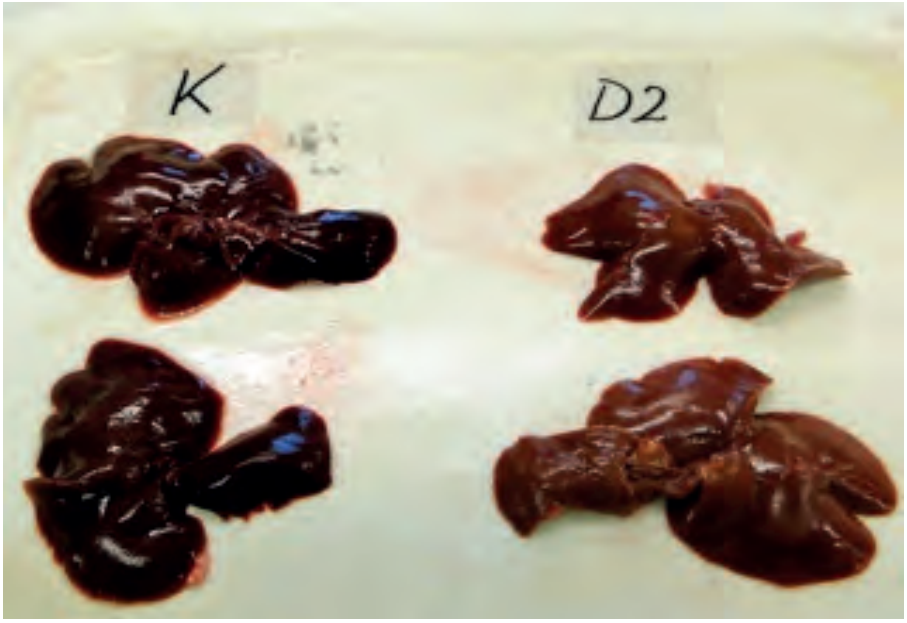
- 116 ppb of Zearalenone.
- Naturally contaminated feed

Feed composition:
(15% DDGS + wheat bran, corn, barley..)

Results (Liver status):

Livers from animals fed with **Escent®** and contaminated feed were less saturated, sharper in colour and lighter.

K (with Escent®) **D2 (without Escent®)**



Age (days)	Statistical analysis n	Group K (with Escent S) 26	Group D2 (without Escent S) 26	
35	x	961	950,06	
	s	104,31	96,34	
42	x	1 330,44	1 250,38	
	s	144,34	135,84	
49	x	1 692,00*	1 561,25*	
	s	192,62	183,65	
56	x	2 065,31**	1 852,63**	
	s	200,45	189,43	
63	x	2 397,50**	2 131,25**	
	s	215,61	223,1	
70	x	2 730,00**	2 444,38**	
	s	227,42	246,2	
77	x	3 004,25**	2 666,88**	+12%
	s	197,14	260,25	

* P≤0,05 ** P≤0,01 x – average s- standard deviation

12% growth improvement at the end of the experiment . Statistically significant.

Conclusions:

- When formulating with DDGS, the risk of encountering possible contamination of mycotoxins do exists.
- Zearalenone detected at a level of 114 ppb has a significant and negative impact on body weight gain of rabbits.
- The use of **Escent®** to counteract such negative toxic effects resulted in significantly better growth (+12% p<0,01) and less oversaturated yellowish liver, indicating a better liver health status

Escent® is not available in the USA and Canada.

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.

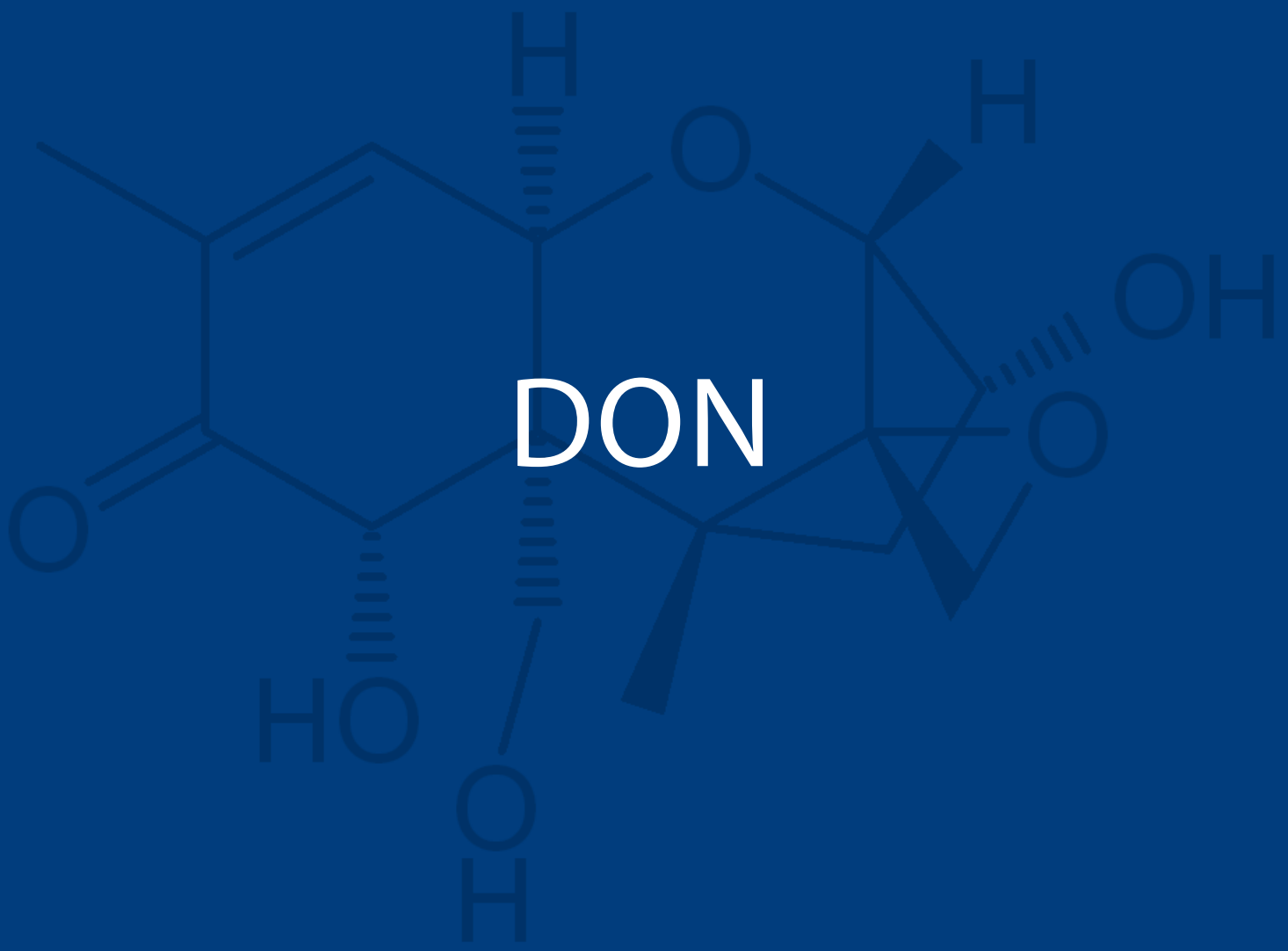
Milk Production:

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.

Escent® is not available in the USA and Canada.



DON

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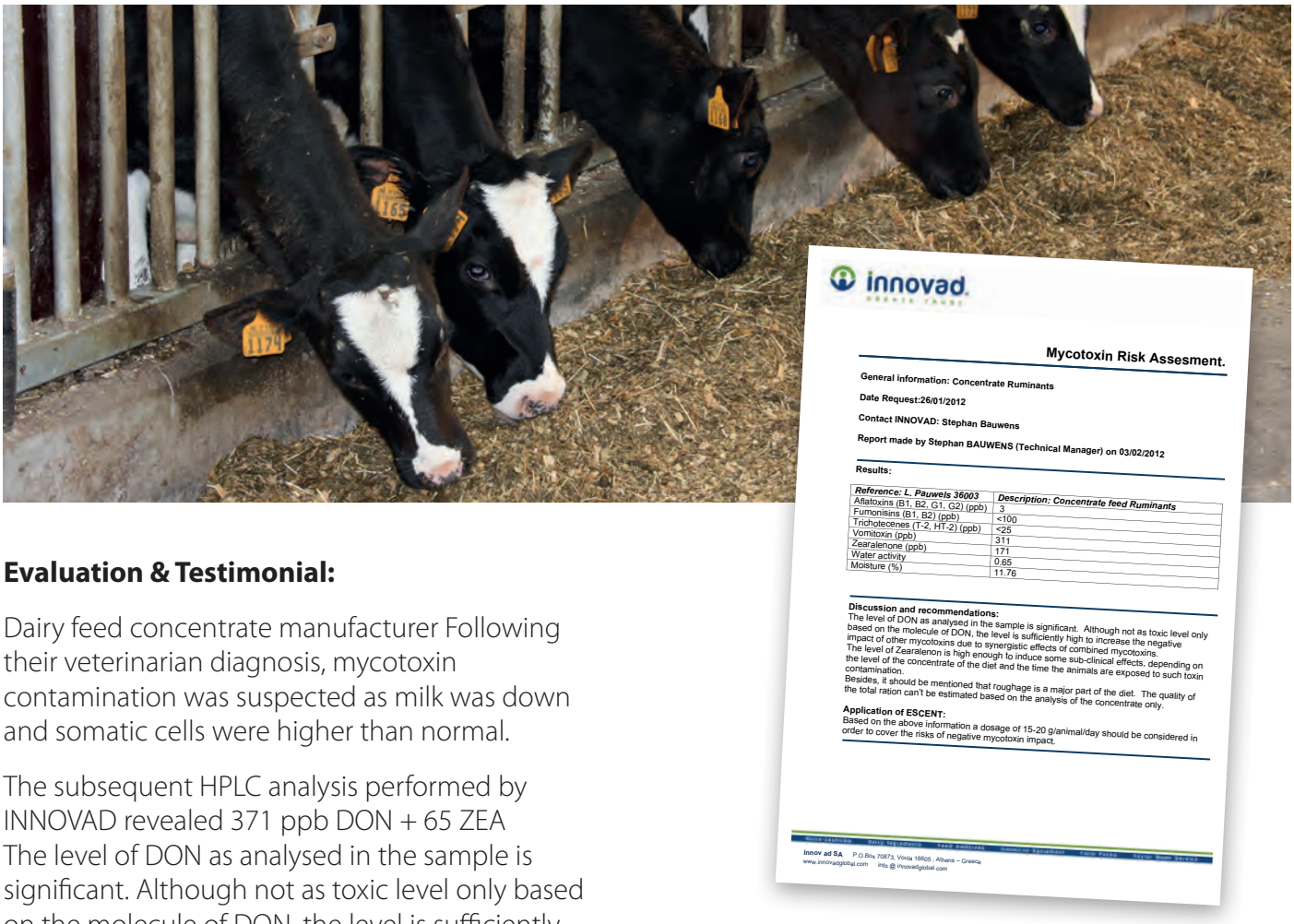
Dairy Evaluation and Testimonials – DON & Zearalenone

Date & Location: January 2012, Belgium **Species:** Dairy Cows



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Mycotoxin Risk Assesment.

General information: Concentrate Ruminants

Date Request:26/01/2012

Contact INNOVAD: Stephan Bauwens

Report made by Stephan BAUWENS (Technical Manager) on 03/02/2012

Results:

Reference: L. Pauwels 36003	*Description: Concentrate feed Ruminants*
Aflatoxins (B1, B2, G1, G2) (ppb)	3
Fumonisins (B1, B2) (ppb)	<100
Tricholecenes (T-2, HT-2) (ppb)	<25
Vomitoxin (ppb)	311
Zearalenone (ppb)	171
Water activity	0.65
Moisture (%)	11.76

Discussion and recommendations:
The level of DON as analysed in the sample is significant. Although not as toxic level only based on the molecule of DON, the level is sufficiently high to increase the negative impact of other mycotoxins due to synergistic effects of combined mycotoxins.
The level of Zearalenon is high enough to induce some sub-clinical effects, depending on the level of the concentrate of the diet and the time the animals are exposed to such toxin contamination.
Besides, it should be mentioned that roughage is a major part of the diet. The quality of the total ration can't be estimated based on the analysis of the concentrate only.

Application of ESCENT:
Based on the above information a dosage of 15-20 g/animal/day should be considered in order to cover the risks of negative mycotoxin impact.

Innov ad SA P.O.Box 70873, Voula 16605 , Athens – Greece
www.innovadglobal.com info @ innovadglobal.com

Evaluation & Testimonial:

Dairy feed concentrate manufacturer Following their veterinarian diagnosis, mycotoxin contamination was suspected as milk was down and somatic cells were higher than normal.

The subsequent HPLC analysis performed by INNOVAD revealed 371 ppb DON + 65 ZEA
The level of DON as analysed in the sample is significant. Although not as toxic level only based on the molecule of DON, the level is sufficiently high to increase the negative impact of other mycotoxins due to synergistic effects of combined mycotoxins.

The level of Zearalenon is high enough to induce some sub-clinical effects, depending on the level of the concentrate of the diet and the time the animals are exposed to such toxin contamination.

Besides, it should be mentioned that roughage is a major part of the diet. The quality of the total ration can't be estimated based on the analysis of the concentrate only.

Application of Escent®:

Based on the above information a dosage of 15-20 g/animal/day should be considered in order to cover the risks of negative mycotoxin impact.

Results (Milk production):

**Producer satisfied (milk production improved and somatic cell counts down).
Re-ordering ever since**

Escent® is not available in the USA and Canada.

The impact of mycotoxin contaminated feed (DON, Trichotecenes T-2) on the performance and liver health status of broilers

Date & Location: October 2011, Northern Europe **Species:** Broilers

Introduction:

Trial design

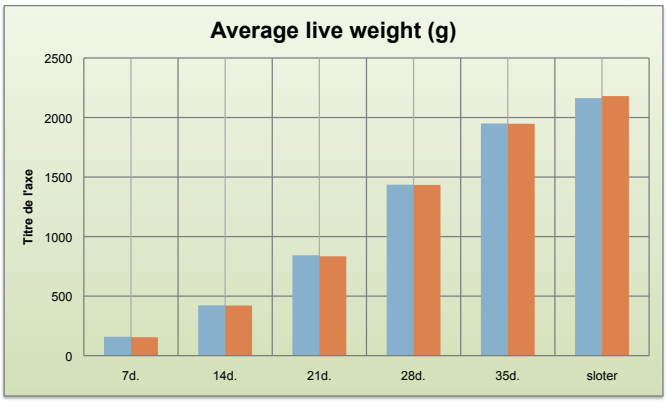
- 26 000 birds per treatment group
- From 1- 40 days
- 2 treatments :
  1. **Escent®**
  2. Competing product "C")

- The presence of the different mycotoxins in the group of the contaminated diet and the **Escent®** group was obtained by the selection of naturally contaminated raw materials.

  The analysis of the contaminated feed resulted in:

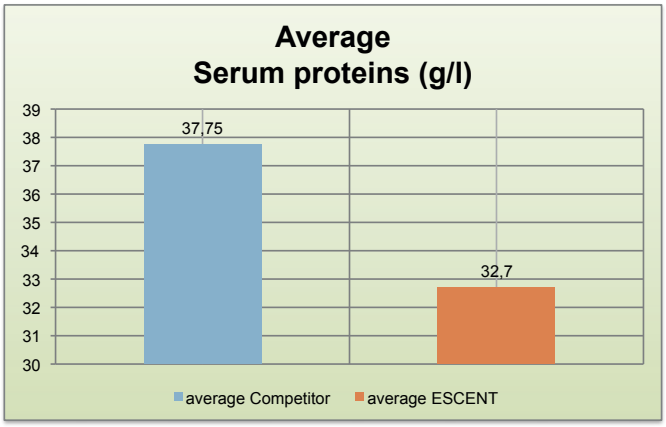
  - **1000-1200 ppb of Deoxynivalenol**
  - **180-250 ppb Trichotecenes T-2**
  - **Escent®** dosed at 1,5 kg/T
  - Thorough blood serum thorough analysis

Results (Body weight):

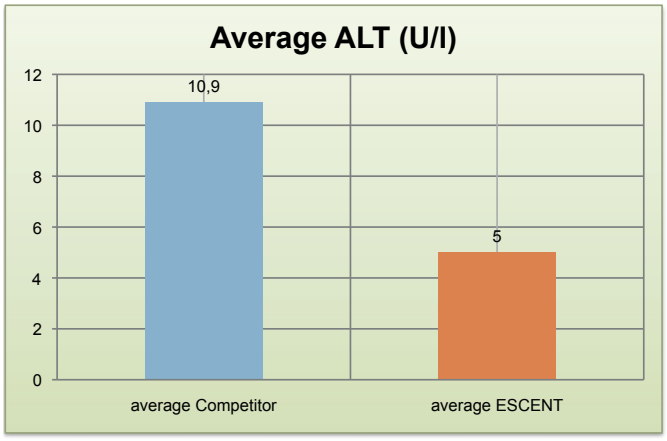


No statistical significance in terms of performance, gain, FCR

Results (Serum Protein):



Total Serum indirectly indicates the functional status of liver and kidney. Elevated protein level can be a consequence of body dehydration, kidney damage, inflammatory reactions to infections



ALT **(Alanine transaminase)** is a hepatocyte (liver's cell) intracellular enzyme found in the cytoplasm. Liver injuries (from toxins or diseases) result in the release of ALT into the blood. Increased level of ALT is the most sensitive indicator of liver dysfunction. (liver necrosis, hepatitis…)

Conclusions:

The examined parameters of the blood serum allow making a decision about the **status of the liver at the cell level**. Equal quantities of DON & T-2 in the feed affected blood serum indicators of the two test groups in a different way. The broilers that received 1.5 kg/t of **Escent®** showed better blood serum indices, which indicates a smaller functional disturbance of the liver and other tissues parenchyma.

Escent® is not available in the USA and Canada.

Scientific Experiment – Pigs

Mycotoxins inactivator evaluations & study. The assessment of the effectiveness of Escent® S on pig mycotoxicosis

Date & Location: May-June 2012

Federal Government Institution – Federal Center for Toxicological, Radiation and Biological Safety – Russia

Experiment Protocol:

Treatments:

4 groups of 10 weaned piglets each.

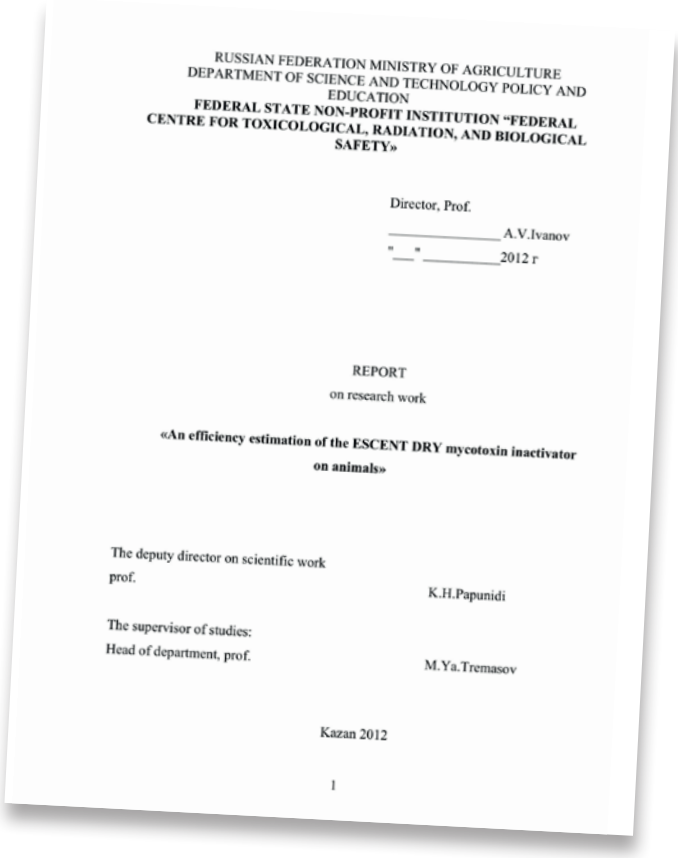
- **Treatment 1:** standard diet free from mycotoxins
- **Treatment 2:** standard diet free from mycotoxins + **Escent® S** at 2kg/ton
- **Treatment 3:** standard diet + 50 ppb of Zearalenone + 1000 ppb DON + 70 ppb T2-toxins (crystal mycotoxins used. Artificial contamination)
- **Treatment 4:** standard diet + mycotoxins + **Escent® S** at 2kg/ton

Duration of experiment:

45 days (2 weeks of preliminary feeding with mycotoxin-free standard diet) + 30 days according to the experiment schedule

The investigated parameters:

1. **Zootechnical** (at 31 days):
   Animal average daily gains, feed conversion
2. **Hematological** (at the beginning, and at 11, 21, 31 days of experiment): Erythrocytes, hemoglobin, hemocrite, erythrocytes mean volume, hemoglobin mean value in erythrocyte, erythrocyte distribution width, leukocytes, leukocytes number, leukocytes percentage, monocytes percentage, monocyte percentage, granular leukocyte percentage, granular leukocyte number, plaque.
3. **Biochemical** (blood sera) at 1 day and at 11, 21 and 31 days of experiment.
4. **Others:** common protein, sugar, albumin, potassium, calcium, common bilirubin, direct bilirubin, urea, common cholesterol, amylase, alaninaminotranspherase, aspartate aminotransferase, alkaline phosphatase, urinary acid.
5. **Histology** – at the end of the experiment (at 31 days of experiment) 3 piglets from each animal group (checked for histology of liver, spleen, kidney, spermary, stomach, intestine) + liver weight + liver photo.
6. **Detection of antibodies** production activity with animal's preliminary vaccination at the beginning of the experiment and antibody titers indication in blood sera at the end of experiment.
7. **Indicator of peroxidation:** the content of malonic aldehyde in the blood (at the beginning and at day 11, 21, 31 days of experiment), vaccination of animals and the identification of antibody activity (at 31 days of experiment).



RUSSIAN FEDERATION MINISTRY OF AGRICULTURE
DEPARTMENT OF SCIENCE AND TECHNOLOGY POLICY AND EDUCATION
FEDERAL STATE NON-PROFIT INSTITUTION "FEDERAL CENTRE FOR TOXICOLOGICAL, RADIATION, AND BIOLOGICAL SAFETY»

Director, Prof.
_____ A.V.Ivanov
"__" _____2012 г

REPORT
on research work

«An efficiency estimation of the ESCENT DRY mycotoxin inactivator on animals»

The deputy director on scientific work
prof. K.H.Papunidi

The supervisor of studies:
Head of department, prof. M.Ya.Tremasov

Kazan 2012

1

Mycotoxins inactivator evaluations & study.
The assessment of the effectiveness of Escent® S on pig mycotoxicosis

(continued from page 23)

Results:

On all parameters studied, there was a clear significant and quantified toxins negative effect and clear positive significant **Escent® S** effect.

Hematological and biochemical parameters

Mycotoxins affect organs and systems. On the first day of the experiment, and every 10 days afterwards, animal blood samples were taken in order to study their hematological and biochemical parameters. The number of erythrocytes, leukocytes, lymphocytes, monocytes, granular leucocytes, hemoglobin, and thrombocytes were evaluated. The level of whole protein, bilirubin, glucose, cholesterol, activity of enzymes alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in animal blood serum was also evaluated.

- Increased **leukocytes** due to mycotoxins presence were reported, along with infections and signs of diarrhea. No increase in the **Escent® S** group. After 10 days the number of leukocytes increased by 48,5% in the intoxicated group versus 6,44% in the **Escent® S** group.
- All **blood parameters** indicate a toxic liver charge and a protective action of **Escent® S**.
- Glucose reduction and bilirubin increase were recorded. The bilirubin level considerably increased with the animal intoxicated group (+ 40.8 %) versus 19.2 % in the **Escent® S** treated group. That evidences the animal liver toxic load but also shows the protective action of **Escent® S**.

Oxidative stress

One of the negative influence mechanisms of mycotoxins on human and animal organisms is the increase of lipid peroxidation. Lipid peroxidation is a normal metabolic process in all organs and tissues; it plays a crucial role in physiological and biochemical cellular homeostasis and also acts as a universal nonspecific link in the mechanism of various pathology developments within the organism. Therefore we evaluated the level of malondialdehyde in blood, as an indicator of increasing lipid peroxidation.

Malondialdehyde levels in pig bloods

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

The results of the evaluation of the lipid peroxidation level (Malondialdehyde) in animal blood show the **Escent® S protective effect against lipid peroxidation** increase due to the influence of mycotoxins.

Immunity

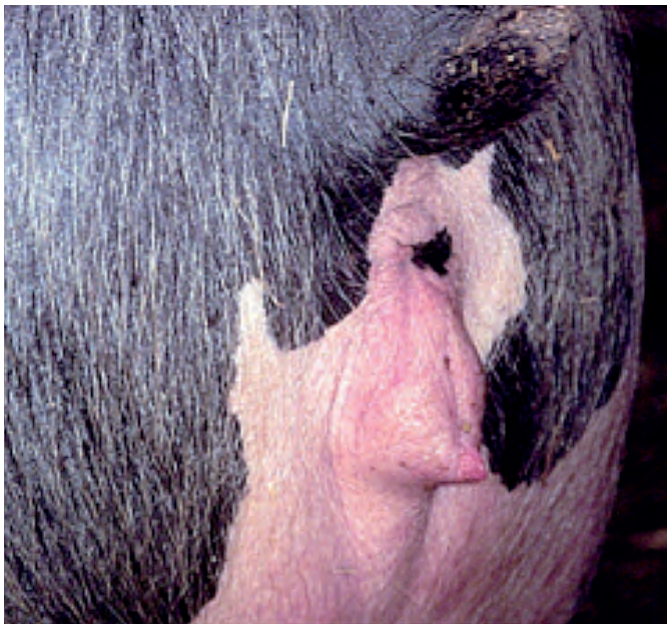
A number of investigations report that mycotoxins suppress immunity. We studied the level of T- and B- lymphocytes in blood. We also studied the immune response of piglets to swine fever vaccination.

- **Antibody titers** response against swine fever vaccination was better with **Escent® S** than the contaminated group.

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

Animal welfare & feed refusal

- Some 3-4 days **Feed refusal** was observed in the contaminated group. Not in **Escent® S**. Animals from the group consuming toxic feed had lower appetite that other groups. During 6-8 days the animals partially refused feed which lasted 3-4 days. Moreover, animals started eating feed again but in smaller volume and pigs were much less active in comparison with the other groups. The reason might be linked to the presence of deoxivalenol in the animal's diet.



- **Gastrointestinal disorders**, vulva swelling, vagina fall back were reported in the contaminated group and not in the **Escent® S** group. Due to prolonged exposure to zearalenone and other mycotoxins, due to young age and animal weakness from infection challenges, *E.Coli & Clostridium* cases were observed.
  Not in the **Escent® S** group.

Escent® is not available in the USA and Canada.

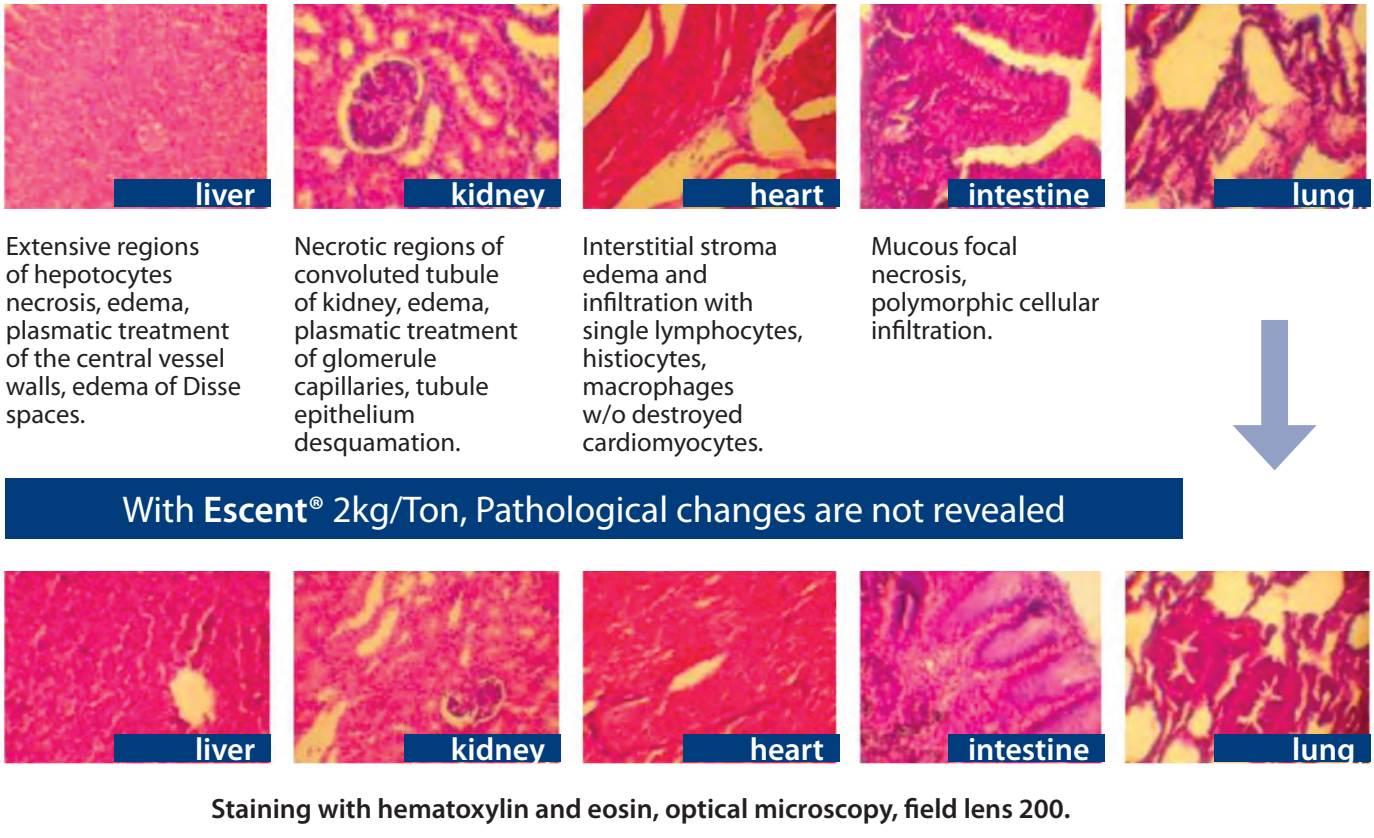
Mycotoxins inactivator evaluations & study.
The assessment of the effectiveness of Escent® S on pig mycotoxicosis

(continued from page 25)

Organ Histology

- Liver weight coefficient (liver weight / body weight) improved with **Escent® S** (from 0,034 to 0,029)
- Microscopic pictures of liver, kidney, spleen, heart, lungs showing evidence of damage by toxins and **Escent® S** protection.

Parameter	Toxic	Toxins + Escent	Clean	Clean + Escent
Body weight, kg	19,326 ±0,39	21,401 ±0,34	22,637 ±0,32	21,435 ±0,33
Liver weight, g	657,084 ±24,37	620,629 ±14,62	611,199 ±19,48	685,920 ±16,51
coefficient	0,034 ±0,001***	0,029 ±0,001	0,027 ±0,001	0,032 ±0,001**

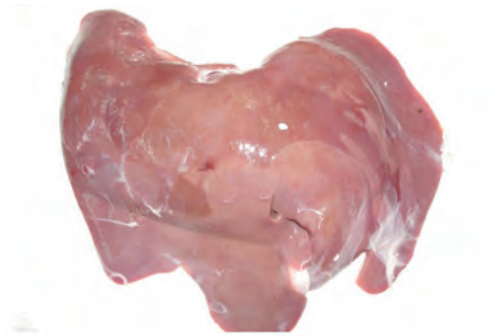


Staining with hematoxylin and eosin, optical microscopy, field lens 200.

The histological studies revealed that the absorption of contaminated feed with mycotoxins results in pathological changes in organs and genital glands.
Liver protein dystrophy. **Kidneys** developed protein dystrophy with epithelial cells desquamation in tubule lumens. Focal necrosis of tubule epithelium. Focal serous edema of interalveolar septum was observed in **lungs**. Pauperisation of white pulp was detected in the **spleen**. The **Dodecadactylon** wall had reinforced polymorphic cellular infiltration with lymphocytes discharge from intestinal

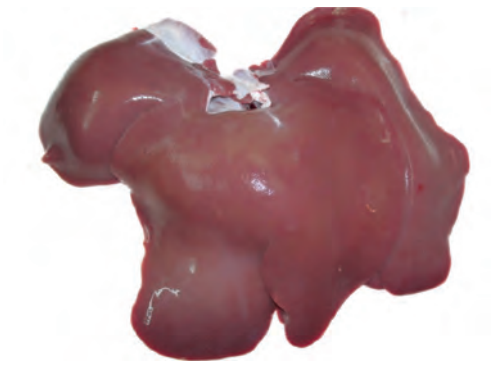
follicle and mucus focal necrosis appeared. The **Gastric wall** had lymphoid cells infiltration and mucus focal necrosis. In the **heart** small regions of interstitial serous edema scarcely expressed polymorphic cellular reaction including lymphocytes, histiocytes, and macrophage cells. The **Ovary** had follicles with uneven colouring of structure.

Piglet liver



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

Contaminated liver



Contaminated feed + Escent® liver

Zootechnical performances

- **Average Daily Weight from** 148,3g (toxins) to 194 g (**Escent® S** + toxins)
- **Consistent feed intake** (26,6 kg in contaminated feed + **Escent® S**) vs. 22,6 kg in contaminated.

Parameter	Toxic	Toxins + Escent	Clean	Clean + Escent
Body weight at the 1st day of experiment, kg	14,65 ±0,22	15,2 ±0,21	14,9 ±0,22	14,86 ±0,23
Body weight by the end of experiment, kg	19,1 ±0,64***	21,02 ±0,40*	22,22 ±0,36	21,79 ±0,37
Weight gain at 30 days, kg	4,45	5,82	7,32	6,93
Average daily gain, kg	148,3	194,0	244,0	231,0
Feed consumption for 1 herd during 30 days, kg	22,6	26,6	26,0	26,8
Feeding conversion	5,07	4,06	3,55	3,86
Animal livability rate, %	80	100	100	100

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

Conclusions:

This research about mycotoxicosis in pigs with the study of hematological, biochemical, immunological, histological, zootechnical parameters indicated that inclusion of **Escent® S** at a dose of 2 kg/t of feed contaminated with mycotoxins has a **strong protective effect** on animals, resulting in normalisation and less lesions.

Escent® S has no negative effects on piglets.

Results suggest that **Escent® S** is safe and effective and can be successfully be used to prevent mycotoxicosis in animals.

Escent® is not available in the USA and Canada.

FUMONISINS

In Vitro Binding of FUMONISIN

Date & Location: USA (Mid West University) – March 2011 **Species:** *in vitro*

Introduction:

Experimental plan
Same as explained before

Methodology & protocol
- 3,000 ppb toxin concentration
- Adsorption pH = 3.0
- Adsorption pH = 6.5

Results : FUMONISIN

% Adsorption at pH 3.0 :	69,40%
% Adsorption at pH 6.0	**76.60 %**

The effect of Escent® on the performance & liver health of broilers fed a diet contaminated with FUMONISINS.

Date & Location: Nov 2010, Brazil **Species:** Broilers

Protocol:

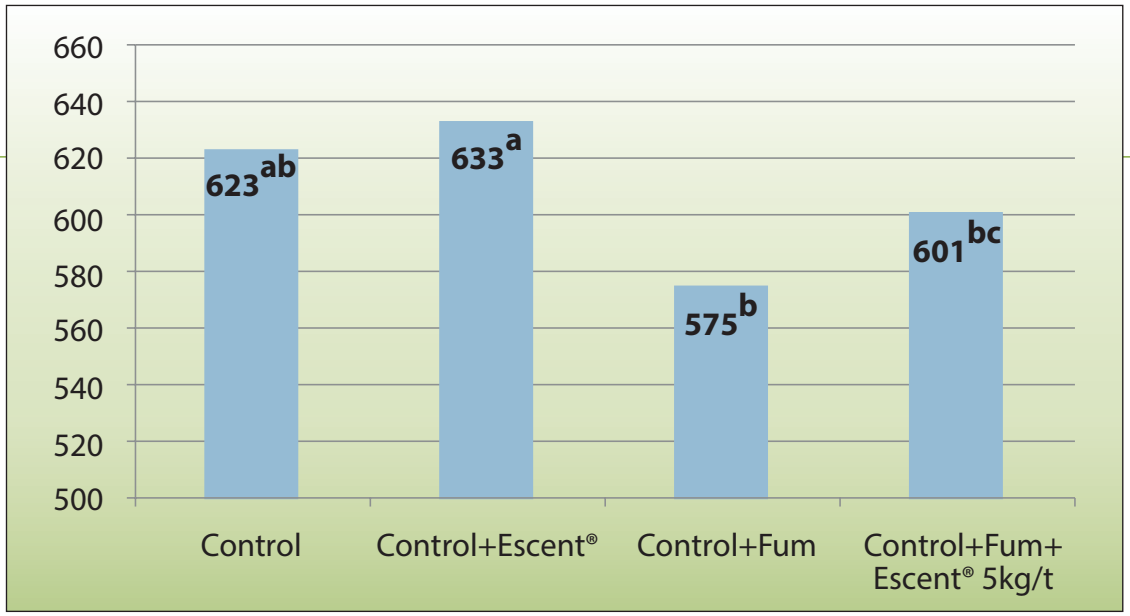
Protocol designed following the guidelines from the Brazilian Ministry of Agriculture for antimycotoxins product registration approval.

- 840 broilers (Cobb 0, 43.3g)
- 7 treat *12 reps * 10 birds - 21 days – ad libitum
- 100 ppm Fumonisins
- **Escent®** at 5 kg/ton of feed

It is recognised that stress levels under field conditions potentiate toxin effects, and therefore may have a negative impact on bird performance even at lower concentrations. Previous studies carried out in Lamic lab showed that the addition of low levels of fumonisins did not produce reliable results, as the observed differences could be attributed to other factors besides fumonisins.

Results (Average weight gain at 21 days)

Statistically significant



INSTITUTO SAMITEC
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RELATÓRIO FINAL DE EXPERIMENTAÇÃO ANIMAL

RESUMO GERAL

Data: 26 de outubro de 2010	Relatório nº: FF04/10

Empresa solicitante:	Auster Nutrição Animal Ltda.
Aditivo avaliado:	Escent P
Espécie animal avaliada:	Frangos de corte
Micotoxinas avaliadas:	Fumonisinas

Início do Experimento:	17 de setembro de 2010.
Término do Experimento:	08 de outubro de 2010.

Parâmetros avaliados:	Peso vivo, consumo de ração, conversão alimentar, peso relativo de fígado, níveis séricos de proteínas plasmáticas totais e relação entre esfinganina e esfingosina (SA/SO).

Avaliação executada em conformidade com as recomendações do Grupo de Trabalho do Ministério da Agricultura, Pecuária e Abastecimento (MAPA) para registro de aditivos anti-micotoxinas.

Trabalho executado em conformidade com a Resolução Nº 879, de 15 de fevereiro de 2008 do CFMV, para experimentação animal e bem-estar animal.

Conclusions:

A clear negative impact of 100 ppm fumonisin on:

- Live weight at 21 days (-7,7%)

The use of **Escent®** (5 kg/ton) in the contaminated diet has a positive effect on:

- Live weight at 21 days (+4,5%)

Escent® is not available in the USA and Canada.

OTHERS

Large Field Evaluation with broilers and natural feed contamination (Ochratoxin, T-2, Aflatoxin, DON)

Date & Location: Quarter 1 2011, Malaysia **Species:** Broilers

Evaluation:

- 6.2 million broilers tested
- **Escent®** 500 gr/Ton
- Ochratoxin : 32 ppb
- Aflatoxin : 19 ppb
- Don : 179 ppb
- T-2 : 62 ppb

Results (Performance):

Average of 3 days gained versus control

Escent® is not available in the USA and Canada.

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The effect of Escent® on the performance and mortality of broilers fed a naturally contaminated diet (low but multiple mycotoxins contamination).

Date & Location: May 2012 – Eastern Europe **Species:** Broilers

Trial design :

They started populating the houses on May 4 and finished it on May 8.

2 houses were fed the treated mix (**Escent®** 1 kg/ton) and the other 2 houses were the control group.

A feed sample was taken for toxin analysis.

- Aflatoxins: 3 ppb
- Fumonisins: < 100 ppb
- T2: 28 ppb
- DON: 72 ppb
- Zea: 55 ppb
- Levels are all quite low but we have a MULTIPLE contamination . The risk is considered low but the level of DON can induce some tight junction leakage at the intestine level, leading to a higher risk of enteritis.

Parameters to be recorded :

feed intake, growth rate, feed conversion, mortality.

Parameter / House	1	2	3	4
Treatment	**Control**	**Escent®**	**Control**	**Escent®**
Starting number of birds	17010	17010	17010	17010
Finishing number of birds	15886	16152	16170	16572
Total weight delivered	34165	36660	37470	41030
Days of life at finish	37,73	37,9	42,77	42,08
Weight at finish (Kg)	**2,15**	**2,27**	**2,32**	**2,48**
		+5,5%		+6,8%
F.C.R	**1,73**	**1,64**	**1,92**	**1,82**
		94,8%		94,7%
Mortality	1124	858	840	438
Mortality %	**6,60%**	**5,04%**	**4,93%**	**2,50%**
Feeding days	599394	612168	691650	697374
Total Feed Consumption (kg)	59260	60340	72180	75060

Conclusions:

Less mortality, more weight delivered, shorter fattening period, higher finishing weight and better feed efficiency.

Even with such a low toxins risk, **Escent®** has the ability to improve performance and health and to reduce mortality thanks to its unique mode of action combining action against oxidative stress, liver support and immunity.

Escent® is not available in the USA and Canada.

Large field evaluation with piglets and natural multiple feed contaminations combined with other bacterial stress

Date & Location: Quarter 4 2012 – Taiwan **Species:** Piglets

Evaluation:

- 100 sows farm
- 3 months observation period
- **Escent® S** dosed at 1 kg/Ton of piglet diet
- **Escent® S** dosed at 0,5 kg/Ton of grower/finisher diet (Corn/soya)
- Multiple Fusarium toxins contamination (Zearalenone, Vomitoxin) combined with additional external stress factors (bacterial challenge)

Results:

Performance

- 3-4 kg higher BW at growing stage
- 7-9 kg higher BW at slaughter
- Less feed for higher LW (FCR)
- Better uniformity

Immune status

- Significantly less symptoms of Porcine Circovirus
- Less respiratory problems
- Scouring problem solved

Economics

- Reduced cost of application of **Escent® S** compared to competitor
- Less medication, especially in transition nursery -> grower stage
- Higher Live Weight and better FCR

Escent® is not available in the USA and Canada.

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*

Escent® is not available in the USA and Canada.



Technical Bulletins & Published Articles



1. DON predisposes to the development of **bacterial enteritis** in both pigs and broilers.

2. The importance of bird **liver function** under low or identified TOXINS contamination in POULTRY feed.

3. TOXINS, the hidden menace in feed & silage and their consequences on **dairy performance**, oxidative stress and liver function.

4. **Physiological disturbance caused by mycotoxins**

5. **Toxic contamination effect on rumen and liver function**

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

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1. DON (Deoxynivalenol) predisposes to the development of bacterial enteritis in both pigs and broilers.

Subclinical enteritis is an economically important enteric disease caused by Clostridium Perfrigens, a gram-positive bacterium. It has been observed by various researchers (G. Anthonissen, Siska Croubels , 2012) that the Fusarium mycotoxin deoxynivalenol (DON) , a common feed contaminant, may damage intestinal epithelium cells and/or their intercellular junctions, subsequently inducing protein leakage (Girish & Smith, 2008), thus predisposing to the development of Bacterial enteritis.

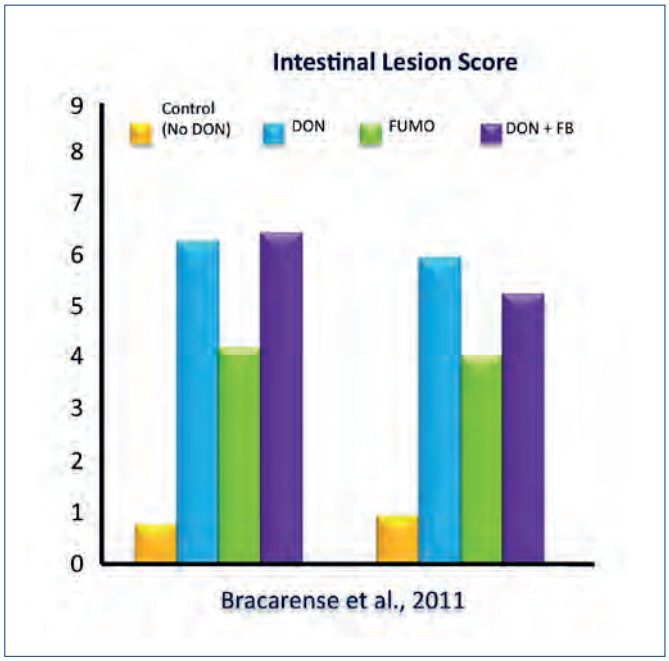
Piglets

Deoxynivalenol (DON) and fumonisins (FB) are the most frequently encountered mycotoxins produced by *Fusarium* species and most commonly co-occur in animal diets. These mycotoxins were studied for their toxicity in piglets on several parameters including plasma biochemistry, organ histopathology and immune response.

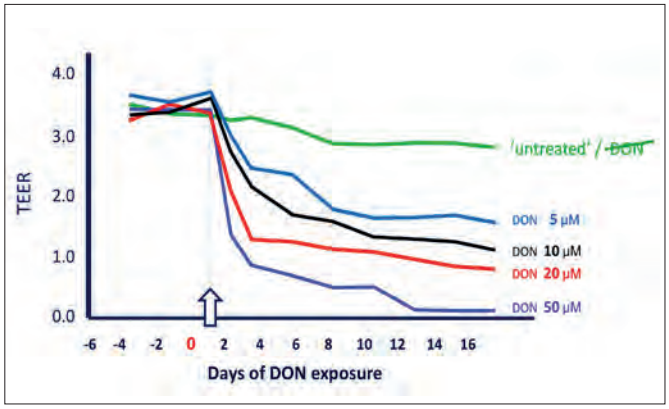
Chronic ingestion of low doses of DON and Fumonisin (alone or in interaction) mycotoxins changes the intestinal structure, and **predisposes animals to infections by enteric pathogens.**

Porcine intestinal cells

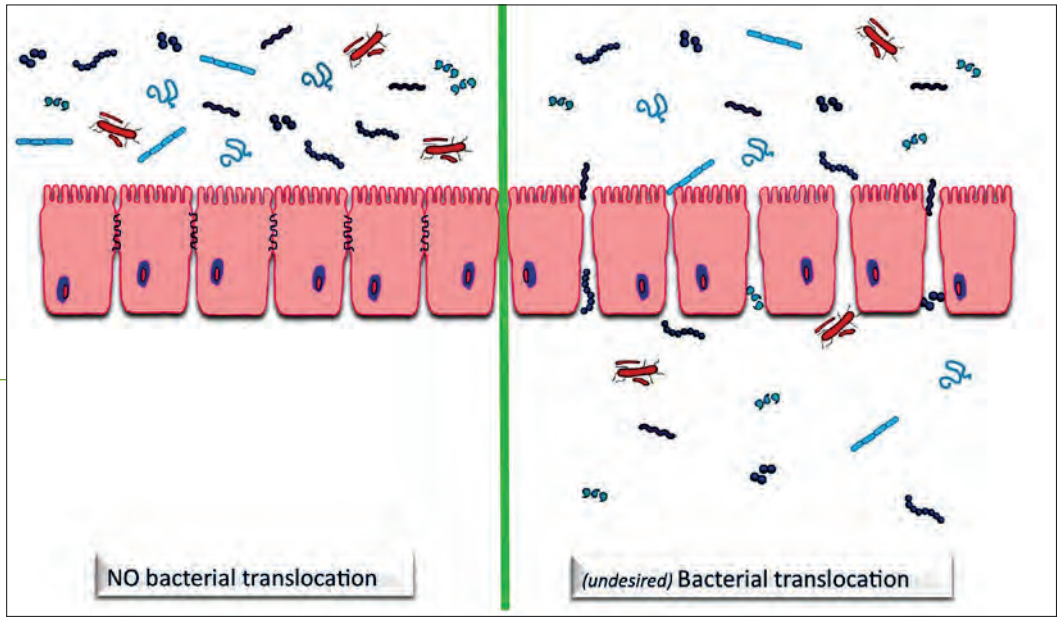
Tight junctions are composed of transmembrane components (proteins) that mediate adhesion and form the paracellular diffusion barrier. Permeability of tight junctions is generally determined by measuring transepithelial electrical resistance (TEER).



DON causes a reduction in TEER of intestinal epithelial cell monolayers.



In intestinal epithelial cells, DON modulates the paracellular pathway, **leading to an increased passage of bacteria.**



Experiment in Broilers

Location:
Department of Pathology, Bacteriology & poultry diseases, Faculty of veterinary medicine, Ghent University, Belgium

Design:

- Control: Clean broiler diet
- Treatment: Contaminated broiler diet:
  a. 3761 ppb (starter)
  b. 4281 ppb (grower)
  c. 4384 ppb (finisher)

Both diets challenged with *C. Prefrigens* $4*10^8$ for 4 days, orally.

Results:

- Control: No lesions
- Control + *C. Prefrigens*: 19,5% lesions (P<0,001)
- Don Contaminated + *C. Perfrigens* : **46,6% lesions**

Chickens that received DON and *C. Perfrigens* had significantly more lesions than chickens that received only *C. Perfrigens*. We can conclude that DON contaminated feed in concentration lower than the maximum guidance contamination level of 5000 ppb to broilers is a **predisposing factor for the development of NE.**

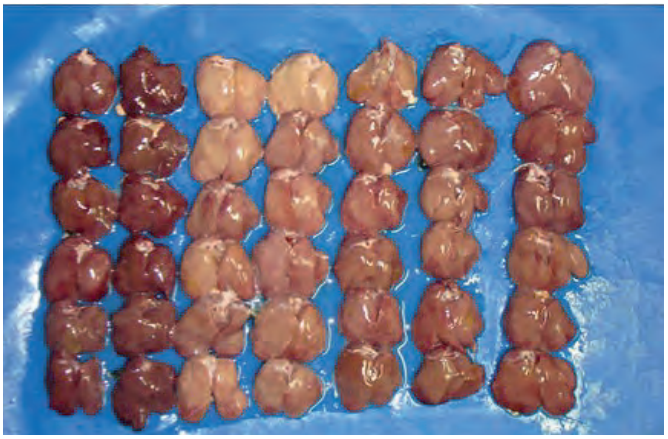
2. The importance of bird liver function under low or identified TOXINS contamination in POULTRY feed.

Detoxification & the role of the liver

Mycotoxins decrease the function of organs such as the liver and kidneys. Mycotoxins are not the only toxic material that the animal has to cope with. The liver, the main detoxification organ, needs to clear and detoxify not only mycotoxins present in the feed, but also enterotoxins (toxins produced by bacteria – that are usually not checked for) and many other contaminants.

Hepatic bioconversions of mycotoxins will need to take place – **risking liver overload** – to change the polarity. The **liver may not be able to detoxify all those components properly**.

Therefore, products that stimulate organ function can reduce the negative impact of the toxins. Various plant extracts, present in **Escent®**, have been known to maintain and restore organ function in case of toxic stressors.



Group 1-2: livers from birds fed non-contaminated feed.
Group 3-4: livers from birds fed mycotoxins contaminated feed
Group 5,6,7: livers from birds fed contaminated feed with **Escent®** at different doses

Oxidative stress

Considering that mycotoxins are among the stress factors that have a negative effect on pro and antioxidant balance in the body and especially in the cell, free radicals can get out of balance. This may lead to a situation where **the bird is no longer able to quickly detoxify these products, leading to oxidative stress**.

How we can help?

Part of the answer is to build dietary strategies to enhance liver function (as well as kidney function) and to aid in reducing intestinal absorption of toxins and their related oxidative stress.

Escent® : 0,5-2,5 kg/ton

We emphasise the need to be proactive when dealing with mycotoxins. This is one problem that is more easily prevented than remedied.

Escent® is not available in the USA and Canada.

3. TOXINS, the hidden menace in feed & silage and their consequences on dairy performance, oxidative stress and liver function

The risk

There is a good chance that moulds and toxins have crept into silos around the country again this year. The key today is to be aware that there is a potential problem with toxins. It might still be too early to tell how serious the challenge might be but it is important that producers are prepared and that they take preventive measures. From past sampling multiple mycotoxigenic moulds, including Aspergillus, Fusarium, Penicillium were found in corn silage samples during harvest and after ensiling. This suggests the possible presence of **multiple mycotoxins**.

The worst thing that can happen is for the producer to end up in a position where he has to **react to a bad situation. By then, dry matter intake is down, the farm has lost a few weeks of milk, and lots of costly ingredients have gone to waste**.

The following symptoms could be associated with multiple mycotoxins contamination and feed related stress:

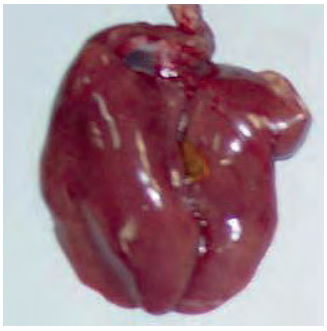
- Below normal milk production
- Oxidative stress
- Increased incidence of disease (opportunistic)
- Reduced production efficacy
- Poor reproductive performance
- Little or no response to veterinary therapy
- Difficulty of diagnosis & masked toxins
- Pale enlarged fatty liver
- Inconsistent dry matter intake

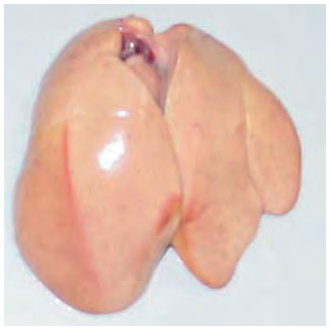
Detoxification & the role of the liver and rumen

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

Besides, such activity is lower in case of rumen dysfunction, or the animal's immune system being depressed (i.e. early lactation). It is then that the cow's liver is affected and needs to convert the toxins into something benign that can be excreted. Hepatic bioconversions of mycotoxins will need to take place – **risking liver overload** – to change the polarity. **Her liver may not be able to detoxify all those components**.

Mycotoxins decrease the function of organs such as the liver and kidneys. Mycotoxins are not the only toxic material that the animal has to cope with. The liver, the main detoxification organ, needs to clear and detoxify not only mycotoxins present in the feed , but also enterotoxins (toxins produced by bacteria – that are usually not checked for) and many other contaminants.
Therefore, products that stimulate organ function can reduce the negative impact of the toxins. Various plant extracts, present in **Escent®** Dairy Pack, have been known to maintain and restore organ function in case of toxic stressors.


normal liver


compromised liver

Oxidative stress

Considering that mycotoxins are among the stress factors that have a negative effect on pro and antioxidant balance in the body and especially in the cell, free radicals can get out of balance. This may lead to a situation where **the cow is no longer able to quickly detoxify these products, 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.
There is a natural balance between freeradical formation and the defense system. For the dairy cow to stay healthy, the system should stay in balance. When the system is out of balance, the body initiates an oxidative chain reaction, resulting in oxidative stress. Peroxide damage can occur in lipids, which can cause free radical formation. Those changes at cellular level can modify metabolic pathways leading to decreased efficiency and premature cell deaths.
Once critical structural damage occurs, antioxidants may no longer be able to repair problems. When free-radicals production is greater than the ability of the animal to quickly detoxify these products, oxidative stress occurs.

How we can help?

Part of the answer is to build dietary strategies to enhance liver function (as well as kidney function) and to aid in reducing intestinal absorption of toxins and their related oxidative stress.

Adding one to two ounces per head per day of **Escent® Dairy Pack** will help. This solution is indicated especially when feed quality and intake isn't what it should be. "More milk is the result of keeping the rumen healthy and the cow functioning normally".

We **emphasise the need to be proactive** when dealing with mycotoxins and moulds. Keep an eye on feed intake. Check the silo regularly. This is one problem that is more easily prevented than remedied.

Escent® is not available in the USA and Canada.

4. Physiological disturbance caused by mycotoxins

Low levels of mycotoxins have a significant negative metabolic and physiological impact on livestock animals. Therefore a multifunctional and preventive approach is crucial to maintain a high level of performance in today's livestock production.

By Stephan Bauwens, Technical Manager, Innovad, Essen, Belgium

Mould can infect almost every agricultural commodity all over the world, during plant growth and/or after harvest. A great variety of these fungi can produce mycotoxins, which can accumulate in raw materials and feed. More than 100 countries nowadays have regulations for maximum levels of mycotoxins, but these regulations do not seem to assure complete safety.

Masked mycotoxins

Plants protect themselves from xenobiotic compounds such as mycotoxins by converting them into more polar metabolites. These metabolites are stored in plant vacuoles or conjugated to structures such as cell wall components. Typical examples of such called "masked mycotoxins" are Zen4G as derivative from Zearalenone, DON 3G and DON 4G as masked forms of Deoxynivalenol, Ochratoxin as conjugate of Ochratoxin A and many more derivatives have been identified over the last few years. Unfortunately, these molecules escape from regular analytical techniques because there is a need for specific procedures for sample preparation. Also, standards of the hidden toxins are not available yet commercially. Recent findings by "Laboratory of Food Analysis" of the University in Ghent indicated a concentration of masked DON varying from 30-98% of the DON level in corn. Very little is known about the availability of the masked mycotoxins in the animals' metabolic system. In 2011 Berthiller was able to demonstrate that 62% of the DON 3G in a diet is transformed back into DON by the microbial population in the large intestine. It would be unwise to ignore this additional and large range of toxins.

Oxidative stress and organ damage

Mycotoxins are incorporated in the cell membrane, affecting its poly-unsaturated fatty acids (PUFA) and leading to detrimental changes in its structure. It is not clear at present if mycotoxins stimulate lipid peroxidation directly by enhancing free radical production or the increased tissue susceptibility to lipid peroxidation is a result of compromised antioxidant system. It seems likely that both processes are involved in this stimulation. In that respect, Abado-Becognee (1998) demonstrated a level of malonaldehyde (MDA), an indicator of tissue oxidation, being more than four times higher in kidney cells in the presence of Fumonisin B1. Considering that mycotoxins are among the stress factors that might create increased oxidation rates in tissues and cell structures, it has to be considered that important organs

Very little is known about the availability of masked mycotoxins in an animals' metabolic system.

such as blood, liver, kidneys, etc. might get affected by the aggressiveness of these molecules. The functionality of the liver, being the largest solid and multitasking organ in the body, is challenged in high performing animal production. It can be easily understood that this threat might lead to metabolic disturbance and loss on performance.

Intestinal integrity

The intestinal tract is the first barrier against ingested antigens, including mycotoxins and pathogenic bacteria. Following ingestion of mycotoxin contaminated feed, enterocytes may be exposed to high concentrations of toxins. Tight junctions play a crucial role in the good functioning of the intestinal barrier and are key when it comes to intestinal integrity. Ana-Paula et al. observed an increased level of inflammatory cytokines (TNF-α and IL-1ß) in the ileum of pigs after ingestion of DON, Fumonisin or a combination of both.

This increased level of cytokines could be linked to tight junction ileal barrier defects and decreased expression of tight junction proteins such as occludin and E-cadherin ***(Figure 1)***. The concentration of the mycotoxins was low and did not affect the technical performance of the animals but did show physiological impact on the intestine. This research confirmed earlier investigations by Pinton et al. ***(Figure 2)*** which observed in vitro a significant reduction of Trans Epithelial Electrical Resistance (TEER) in intestinal epithelial cell monolayers treated with different concentrations of DON. Finally this research concluded that the reduction in TEER is related to a reduced expression of tight junction proteins caused by DON exposure.

Figure 1. The effect of DON, Fumonisin B1 and a combination of both on the expression of the tight junction proteins occludin and E-cadherin. (Source: Ana-Paula et al., 2011)

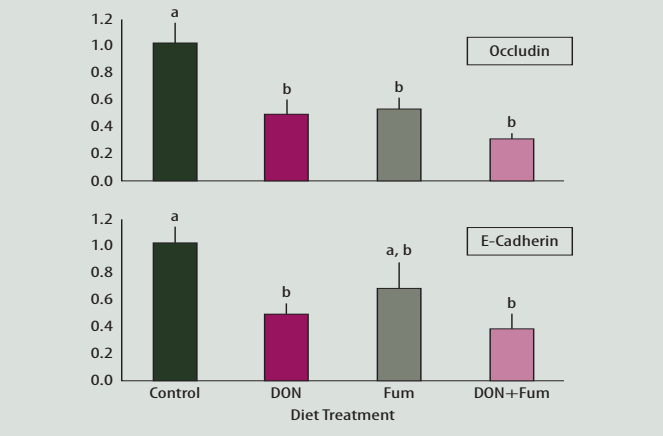


Figure 2. Non-cytotoxic doses of DON (on cellular level) decrease the TEER of porcine intestinal cells in a dose and time dependant manner. (Source: Pinton et al., 2009)

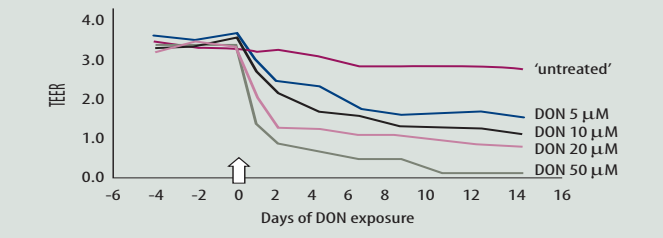
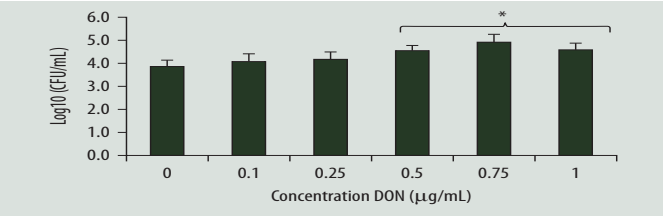


Figure 3. Impact of DON on transepithelial passage of Salmonella Typhimurium refers to a significantly higher translocation of the bacteria compared to the unexposed control cells (p<0.05). (Source: Vandenbroucke et al., 2011)



Bouhet et al. (2003) investigated and concluded similar effects on TEER of epithelium cells when exposed to non-cytotoxic levels of Fumonisin B1.
The above summary of data clearly indicates that current legal limits of mycotoxins do not exclude physiological and metabolic changes which might affect general health and performance.

Double trouble: Mycotoxins and pathogens

Vandenbroucke et al. observed the intestinal and systemic infection phase of *Salmonella Typhimurium* in pigs ***(Figure 3)***. Intestinal cell lines, pretreated with non-toxic levels of DON, showed an increased invasiveness of S.
Typhimurium and an increased translocation through the cell layer. Within an intestinal loop model, it has been demonstrated that simultaneous exposure of the intestinal tract by non-cytotoxic concentrations of DON and *S. Typhimurium* resulted in an increased inflammation, which was not observed when exposed to only DON or *S. Typhimurium*. An in vitro approach as model for the systemic phase of the infection revealed, at low concentrations of DON, an enhanced uptake of *S. Typhimurium* by the macrophages. It is well known that Salmonella can shelter and multiply in macrophages while being spread throughout the body.
Antonissen et al. (2012) challenged broiler chickens with *Clostridium perfringens*, being fed a control diet or a diet contaminated with DON (< 5000 ppb). Chickens that received DON had significantly more lesions (46,6%) compared to the group challenged by *C. perfringens* without DON-exposure (19.5%). Within the current philosophy of disease prevention, mycotoxin control should become part of the basic strategy.

Better prepare than repair

Part of the answer is to build dietary strategies to enhance liver function (as well as kidney function) and to aid in reducing intestinal absorption of toxins and their related oxidative stress.
Recent data from the Polish University of Warm sko-Mazurski in Olsztyn (Prof Andrzej Gugołek) demonstrated the impact of Zearalenone (with or without treatment of **Escent®**) on the performance of rabbits. Feed, contaminated with 116 ppb of Zearalenone, was used as control diet. Escent was included in the same feed as treatment group. The trial started at day 35 of age and from day 39, body weight was significantly different between both groups (p<0,05), while from day 56 onwards significance was even more pronounced (p<0,01). At the end of the trial, 12% better growth with the **Escent®** group. Dressing % was increased by 4.6% as well as liver colouration.

In Eastern Europe, a trial has recently been carried out where the feed was naturally contaminated with low, but multiple levels of mycotoxin (Aflatoxin 3 ppb - Fumonisin <100 ppb - T2 28 ppb – DON 72 ppb and Zearalenone 55 ppb). **Escent®** has been dosed at 1 kg/tonne. The treatment with **Escent®** resulted in a live weight which was 6.2% higher, a FCR which was 5.2% better and a mortality which was 3.75% compared to 5.8% in the control diet.

From mycotoxins, it is know that they might cause sub-clinical and clinical signs of intoxication. Based on the safety assessments, legal limits have been set forward for different toxins. Today's research clearly indicates we understand only a tiny part of the complex mycotoxin puzzle. New challenges of low and legally allowed levels of mycotoxins do have a significant metabolic and physiological impact on the animals. Therefore a multifunctional and preventive approach is indispensable to maintain the high levels of performance in today's competitive animal rearing. **AAF**

Escent® is not available in the USA and Canada.

Published Article

5. Toxic contamination effect on rumen and liver function.

Stephan BAUWENS – Technical Manager INNOVAD SA/NV (Belgium)

Moulds are filamentous fungi that occur in many feedstuffs including roughages and concentrates. Moulds produce poisonous substances, called mycotoxins, which affect the animal's health and productivity. This disorder is known as mycotoxicosis.

Mycotoxins are produced by a wide range of different moulds and are classified as secondary metabolites, meaning that their function is not essential to the mould's existence. Mycotoxins can be formed on crops in the field, during harvest, or during storage, processing, or feeding. Moulds are present throughout the environment. Their spores are present in high concentrations in the soil and in plant debris, and lie ready to infect the growing plants in the field. Field infestation is characterised by yield loss, quality loss and mycotoxin contamination. Mould growth and production of mycotoxins are usually associated with extreme weather conditions, poor storage practices, low feedstuff quality, and inadequate feeding conditions. Because feedstuffs can be contaminated pre-harvest, control of additional mould growth and mycotoxin formation are dependent on storage management. After harvest, temperature, water activity, and insect activity are the major factors influencing mycotoxin contamination of feedstuffs.

It is generally accepted that the Aspergillus, Fusarium and Penicillium moulds are among the most important mycotoxins producing moulds that are detrimental to cattle.

The potential risk for mycotoxins is mostly well understood for cereals like corn, wheat and barley, but the situation is largely different when we

consider roughages for ruminants, although these feedstuffs often represent the most important part of the diet. Potential reasons for the unknown risk are multiple. Silages are less frequently traded; they are more heterogeneous and present sampling and analytical challenges. An understanding of possible risks, for this part of the diet in particular, is important realising consistent production, protecting animal health and well-being and improving farm economics.

Fusarium infections are more commonly associated with warm conditions at silking, insect damage and wet conditions late in the growing season. It has been found on fresh harvest corn, but no viable spores could be found in the silage. Although Fusarium apparently does not develop inside the silage, Zearalenon, Fumonisins and Deoxynivalenol belong to the most common toxins in ensiled roughages.

The individual Penicillium species have variable requirements for temperature and moisture but are more likely to grow under post-harvest conditions, in cooler climates, in wet conditions, at a lower pH and some require little oxygen. Penicillium moulds produce ochratoxin and silage derived mycotoxins such as Roquefortine C. Several Penicillium species could be detected in fresh corn, while several of these have been analysed at a higher frequency in ensiled material, indicating Penicillium develops inside the silo. Roquefortine C is frequently detected in ensiled corn, adding to the list of most common mycotoxins in silages. The silage derived mycotoxins have an antibiotic effect on rumen microbes, reducing their detoxification capacity. Several other mycotoxins such as the ergots and patulin are known to affect cattle and may be

prevalent at times in certain feedstuffs and silages.

Aspergillus species normally grow in lower water activities and at higher temperatures than the Fusarium species. Therefore, Aspergillus flavus and its related toxin (aflatoxin) in corn are favoured by the heat and drought stress associated with warmer climates.

Ruminants are generally considered to be less susceptible to the adverse effects caused by mycotoxins. For animals with a completely developed forestomach-system, the rumen fluid content is, for certain mycotoxins such as ochratoxin A, zearalenone, T-2 toxin, diacetoxyscirpenol and deoxynivalenol, a detoxifying barrier with protozoa being significantly more active than bacteria. For this reason, the rumen has long been considered a very strong buffer against a possible negative impact of the mould's toxic metabolites. However, other aspects should be taken into account before disregarding the hazardous effect of mycotoxins in ruminants.

Not all toxins are degraded or transformed. Aflatoxin, most known for its transition as Aflatoxin M1 in milk, is hardly transformed and passes the rumen for approximately 90%. The other way around, transformation of mycotoxins in the rumen environment is not always equivalent to reduced toxicity. 90% of Zearalenone (ZEN) can be converted to α-Zearalenol, which is ten times more toxic than its' parent toxin itself.

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 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. Additionally, toxins like Aflatoxin and Deoxynivalenol reduce feed intake and by consequence further nutrient supply.

In dairy cattle, T2-toxin has been associated with intestinal hemorrhages, bloody faeces, gastro-intestinal lesions and enteritis, and finally disrupting the digestive process in the lower part of the digestive tract.

Reproduction and fertility, cornerstones of modern dairy farms' economics, can be compromised significantly by the presence of ZEN. Swollen vulvas, vaginal or rectal prolapse can be observed as well as lower conception rates, mastitis or abortions.

Crucial organs, such as the liver, are stressed and damaged by the aggressiveness of the mycotoxins (Aflatoxin and Fumonisin) after absorption, while immune function is compromised by most of the already mentioned toxins. Both the liver and the immune cells, have a very high metabolic activity which makes them extra vulnerable to oxidative stress by aggressive molecules such as these. Oxidative stress is an imbalanced ratio between free radicals (endogenously or exogenously produced) and the natural existing anti-oxidant system for free radical neutralisation. Considering that mycotoxins are among the most important exogenous stress factors that might create increased oxidation rates in tissues and cell structures, it has to be considered that important organs such as blood, liver, kidneys, etc. might get affected by the aggressiveness of these molecules which results in impaired functionality.

Another aspect worth mentioning is the higher incidence of lameness on dairy farms contaminated with mycotoxins. Lameness alone in dairy farms causes large financial losses due to decreased milk production, impaired reproductive performance and higher culling and veterinary costs. In a study conducted in 2005 by Özsoy S, et al., a positive relationship was established between aflatoxin contamination of feed, lameness (subclinical laminitis) and impaired fertility (cystic ovaries).

The toxins of major concern for dairy cows are Aflatoxin, Deoxynivalenol, Zearalenone, T2-toxin, Fumonisin and PR-toxin. Their negative impact is amplified by a negative energy balance and/or high productive stress, but most of the time starts unnoticed. Within days or weeks, the effect of continued mycotoxin ingestion on performance becomes more pronounced; although symptoms remain very variable and mycotoxin induced diseases seldom respond if at all to veterinary therapy.

Feeding animals with mycotoxin contaminated feed causes a range of problems starting from feed intake to impaired milk production, reduced reproduction, lower immunity and health status. All together, they might be responsible for significant economic losses, by some estimated at billions of dollars.

Research and practical experience have proven that there is no single method for effective mycotoxin control, but many agree that mycotoxin management in the diet is a valid insurance policy. The best way to counteract problems related to mycotoxins might lay in the combination of actives, partially focusing on direct mycotoxin absorption and/or transformation, while ensuring metabolic support at the same time. Metabolic support should put emphasis on maintenance and balancing the rumen micro flora, oxidative stress management, essential organ support and immune stimulation. ■

Escent® is not available in the USA and Canada.

Published Article

6. 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. Moulds and mycotoxins, endotoxins, hidden toxins in the feed, extra heat, pathogens challenges, environmental issues, changes in diets, transition period and calving all compromise the cow's immunity 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.

Moulds 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 mould growth and toxin formation do not necessarily need to coincide.

Moulds 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 indicate 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 cow's production challenges seems to lay in a combination of actions – the cow's metabolic support emphasising 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 the 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.

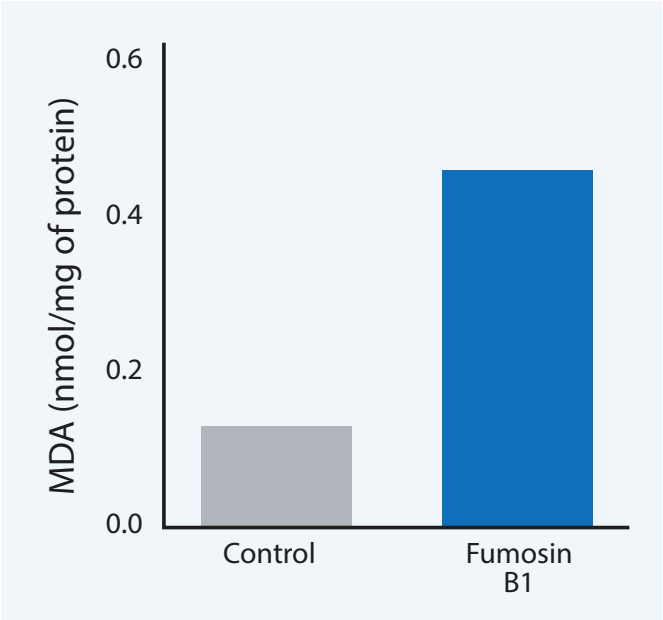


Figure 1. Oxidative activity of fumonisin B1 on kidney cells (Abado-Becognee et al 1998).

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

The antioxidant system of the mammalian cells 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 stabilise the existing system. Polyphenols are a complex group of

substances, which can be divided into phenolic acids and flavonoids, and subdivided much further.
They play an important part 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.

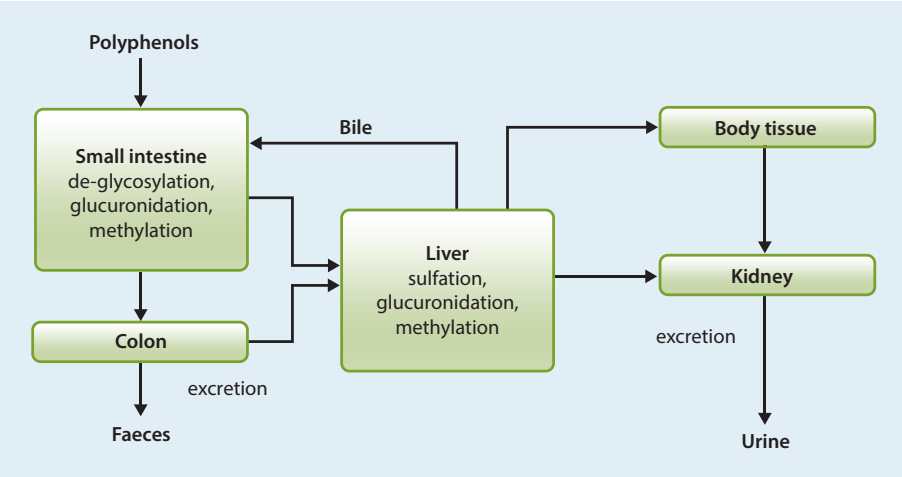


Fig. 2. Metabolism of polyphenols.

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.

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 to 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 compromised 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. Moulds 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 is supported by trials with cell cultures (in vitro model), animal studies (in vivo model) and clinical trials in humans.

Rosemary 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. They are 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

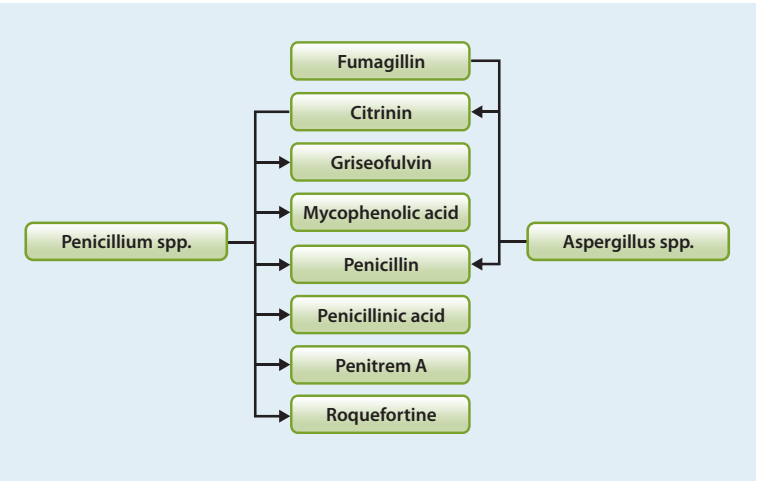


Fig. 3. Antibiotics produced by Penicillium and Aspergillus spp.

matter intake, acid detergent fibre (ADF), starch digestion and on microbial growth are some of the 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.

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

Fermentation extracts can maintain the rumen functioning and performance even in the presence of mycotoxins. They supply micronutrients such as B vitamins, branchchained 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 by 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 the 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 stabilisation of the immune system results in fewer cases of mastitis and a and lower concentration of somatic cell count.

Conclusion

At the beginning of lactation, during high mobilisation 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 stabilise the health of the cow naturally. Innovad's Escent can keep the liver and kidney healthy, as well as the rumen highly productive, resulting in more milk. ■

Escent® is not available in the USA and Canada.

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

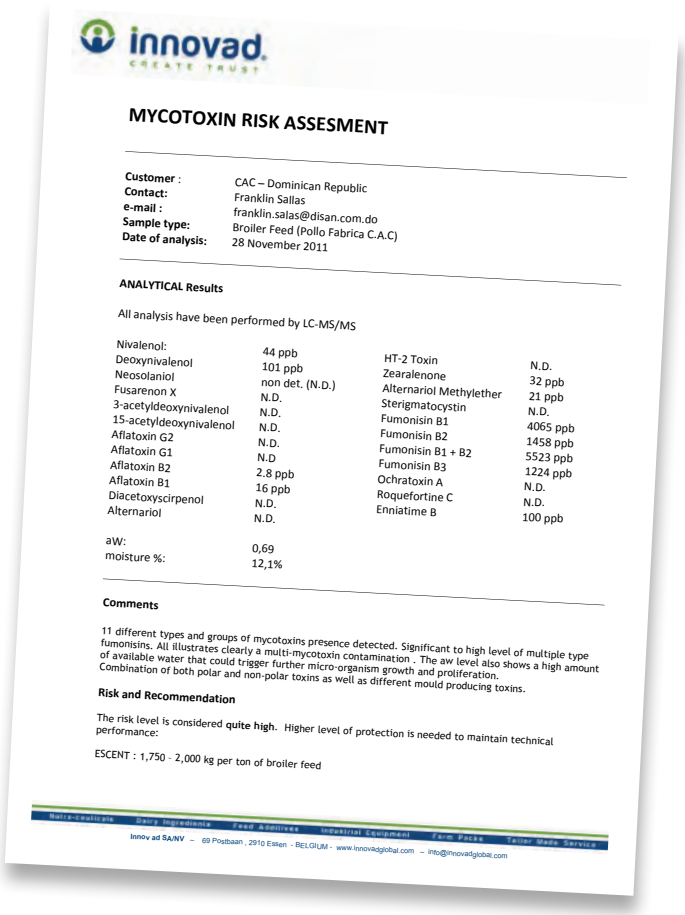
INNOVAD® has developed a specific service to help assess the potential contamination risk and performance losses related to the presence of toxins. Making use of analytical techniques and own database, INNOVAD® offers practical solutions based on accurate recommendations.

Samples are collected and sent for analysis following HPLC or 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
- Analyse 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 environmental 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



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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

Nutri-cosmetics Dairy Ingredients Feed Additives Industrial Equipment Farm Packs Tailor Made Service

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

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Mycotoxin Analysis
Analytical Report

Actlabs Agriculture

Report ID: US15-D-SEP25-003
Received Date: September 25, 2015
Printed Date: September 29, 2015

Innov ad NV/SA
Mike Collins
33 Eagle Drive
Rehoboth, Delaware 19971

Sample ID: X15-001120
Sample Number: 1
Sample Description: [redacted] TMR
Submitted For: [redacted]

Mycotoxin	Value (Dry Basis)	Units	RL	Method ID
Aflatoxin B1	< 1	ppb	1	AGR DI M 1.2
Aflatoxin B2	< 1	ppb	1	AGR DI M 1.2
Aflatoxin G1	< 1	ppb	1	AGR DI M 1.2
Aflatoxin G2	< 1	ppb	1	AGR DI M 1.2
Deoxynivalenol (DON)	1.17	ppm	0.06	AGR DI M 1.2
3-Acetyl-Deoxynivalenol	< 0.06	ppm	0.06	AGR DI M 1.2
15-Acetyl-Deoxynivalenol	0.30	ppm	0.06	AGR DI M 1.2
Fumonisin B1	< 0.1	ppm	0.1	AGR DI M 1.2
Fumonisin B2	< 0.1	ppm	0.1	AGR DI M 1.2
Ochratoxin A	< 0.003	ppm	0.003	AGR DI M 1.2
T-2	< 0.06	ppm	0.06	AGR DI M 1.2
HT-2	< 0.06	ppm	0.06	AGR DI M 1.2
Zearalenone	0.03	ppm	0.03	AGR DI M 1.2
Diacetoxyscirpenol	< 0.06	ppm	0.06	AGR DI M 1.2
Sterigmatocystin	< 0.03	ppm	0.03	AGR DI M 1.2

Results Approved By: Michael Wyrebek

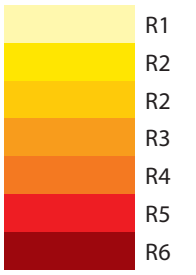
Michael Wyrebek, MSc

Risk assessment

		TMR Farm 1	TMR Farm 2
	Mycotoxins (ppb)	**1120**	**1121**
1	Aflatoxin B1		
2	Aflatoxin B2		
3	Aflatoxin G1		
4	Aflatoxin G2		
5	**Deoxynivalenol (DON)**	1.170	670
6	3-acetyldeoxyvalenol		
7	15-acetyldeoxyvalenol	300	130
8	**TOTAL DON**	1.470	800
9	Fumosin B1		
10	Fumosin B2		
11	Ochratoxin A		
12	T-2 toxin		
13	HT-2 toxin		
14	Diacetoxyscirpenol		
15	Sterigmatocystin		
16	Zearalenone	30	110

Number of mycotoxins
Level Risk

Escent® S
Min dose (g/head/day)



Conclusions:

The main observation is that the level of DON contamination in the TMR represents a risk.
No polar toxins risk. No Afla.
All toxins are of non-polar form, so cannot be bound with simple clays only.
DON and conjugated form of DON and Zearalenone present are of non-polar form. There will be an impact on liver functioning. There will be an impact on gut integrity and intestinal barrier due to the presence of DON and Conjugated DON.
Such chronic and multiple mycotoxin contamination at above levels will impair rumen functioning.
This risk will generate possible inflammatory response.
Protection with Escent® S is strongly recommended at above feeding rates.

Product Data Sheet

ESCENT® S

Scope	Feed quality accounts for the larger part of a successful intensive animal rearing operation. Raw materials and compound feeds require special care in order not to make them a source of mechanisms (poisonous substances, interference with digestibility, oxydation and free radicals) that affect feeding efficiency and animal production response. Such mechanisms can cause severe economic losses to the producer. Carefully selected and proportioned feeding materials and additives help to ensure making quality feedstuffs available to the animals even under the most severe conditions.
Description	Premixture. A well balanced synergistic mixture of carefully selected additives for use in animal feedstuffs.
Components	Hydrated Na-Ca Aluminium silicates, Inactivated yeast and yeast extracts (Saccharomyces cerevisiae), plant derivatives, Calcium propionate, BHT (Butylated Hydroxytoluene), Ethoxyquin, Citric acid
Physical & Technical Specifications	**Appearance** : powder **Colour** : light to dark brown **Specific Gravity** : 0,65 - 0,95 kg/l **pH (10%)** : 5,5 - 6,5 **Moisture** : Max 9,5 % **Colour change or variation does not affect performance.**
Application & Dosage	Feed: 0,5 - 2,5 kg/T
Packaging	Standard 25 kg export worthy bags on wooden fumigated pallets. Other net weights 1 kg*, 5 kg*, 10 kg, 20 kg, 750-1000kg, bulk on demand. *Marked unit packs are packed in an outer carton up to max. 25 kg.
Shelf Life	2 years when stored in a cool and dry environment out of direct sunlight in unopened packing.
Item Reference	**10035**

The information in this data sheet is to the best of our knowledge true and accurate. All instructions, recommendations and suggestions are made under reserve. Since the conditions of use are beyond our control, the manufacturer disclaims all liability for loss or damage suffered from the use of these data, suggestions or recommendations.

Innov ad SA
P.O.Box 70873
Voula 16605, Athens - Greece

PDS/3 DATED 26-05-2011

Anti-stress, mycotoxins control and animal revitalisation solution

Escent® L

in drinking water application

innovad®
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Stress & poultry production issues

Stress is dynamic, complex

In commercial poultry production, increasing resilience has been achieved through breed selection, use of additives and nutrient composition that helps birds to ignore and recover from stress. In poultry management, stress factors such as handling, sudden environmental changes and vaccine and disease challenges are minimized. Any treatment that prevents or minimizes stress translates into growth promotion.

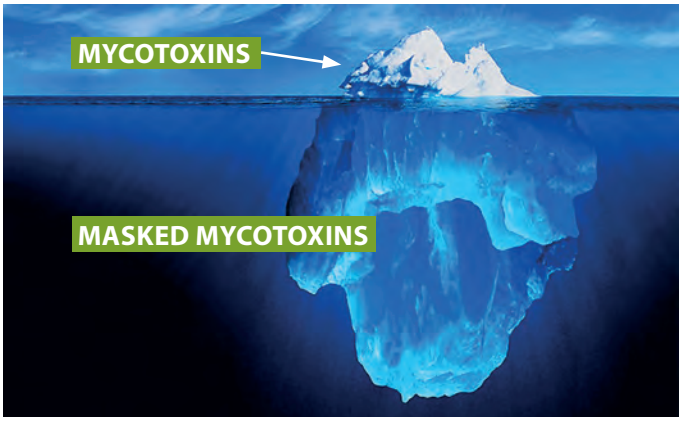
Animals possess a limited natural resistance and immunity against colonization or infection by potentially pathogenic micro-organisms or other toxic components.

Practical and easy applicable dietary or **drinking water applications** to support the immune system are an important tool to address **specific and non-specific stress related situations** in modern poultry production to obtain optimal field performance and body composition of poultry.

Sources of Stress

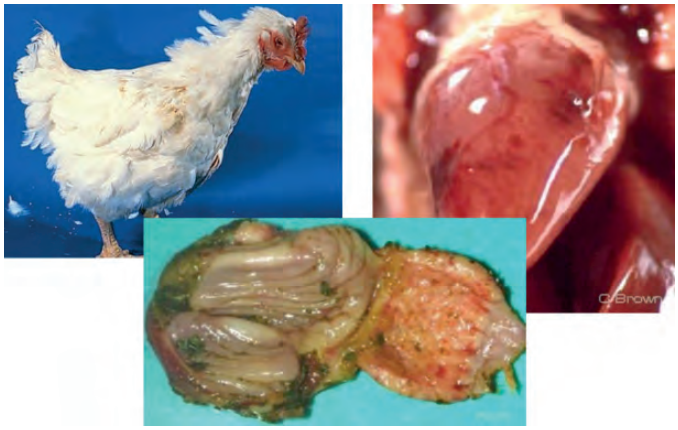
1. Multiple Mycotoxins, hidden toxins and undetected endotoxins

In nature there are more than 400 mycotoxins, but analytical techniques for routine mycotoxins analysis have been developed only for about 30 major mycotoxins.



Sampling for mycotoxin analysis is extremely difficult and is an important **source of errors**. Hence there are no safe levels of mycotoxins, because of synergistic interactions between mycotoxins: Several mycotoxins in low concentrations could cause more problems than a single mycotoxin at a higher dose.

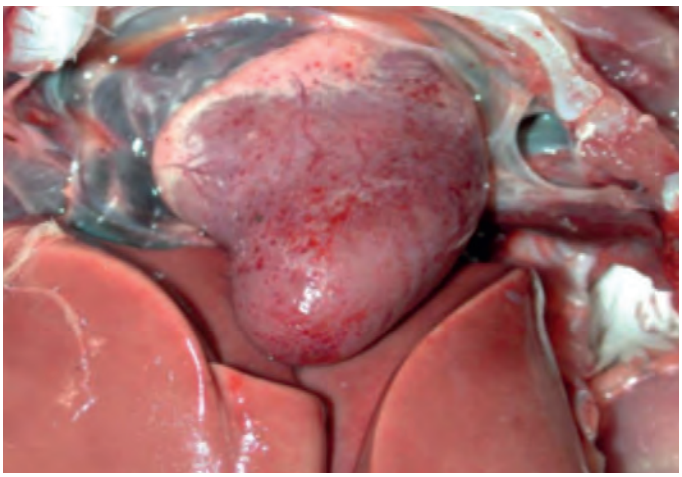
Hidden mycotoxins are more dangerous as they have seldom been monitored. Also, their accumulated effect and synergy at low level can cause severe economical losses for the producers.



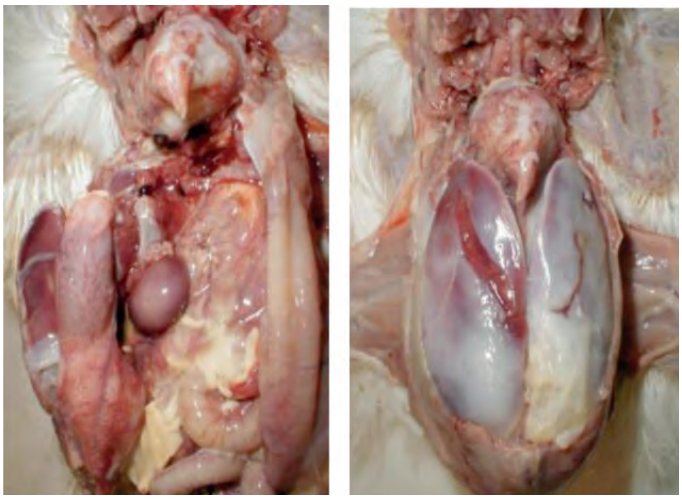
CRD (Chronic Respiratory Disease), Mycoplasma gallisepticum, Mycoplasma synoviae .

2. Old & emerging diseases

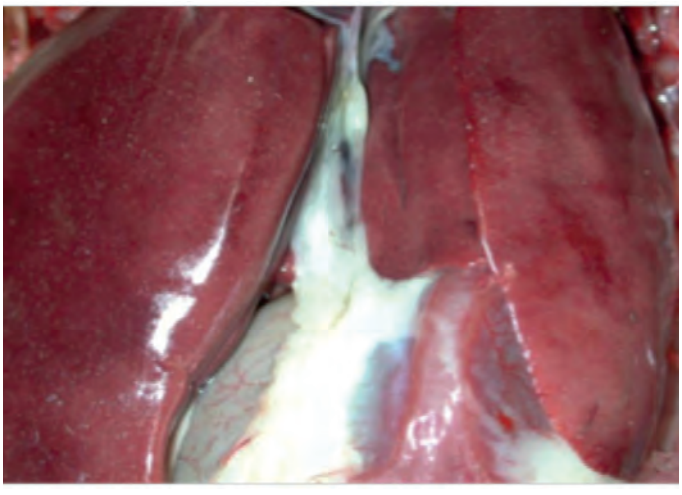
Disease are major concerns within intensive animal production, mycotoxins have a significant negative impact on the animal defense mechanism and immune system.



Petechiae in the heart of goose (pasteurellosis, erysipelas, asphyxia)



Colibacillosis: Gram negative rods, Septicaemia, Coli granulomatosis (intestine, mesenterium)

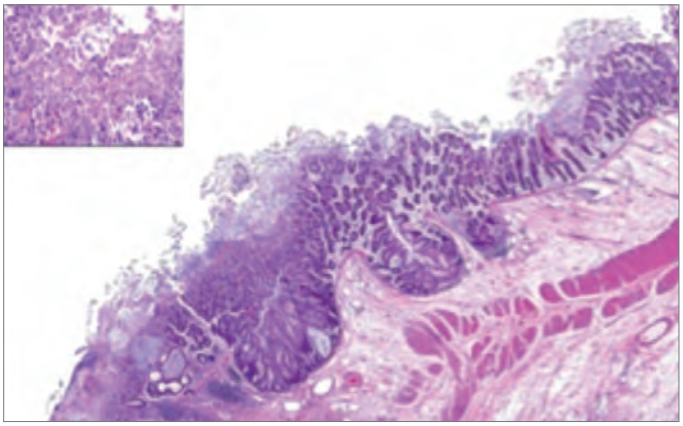


Necrotic foci in the liver goose (pasteurellosis, erysipelas)

Stress & poultry production issues

3. Enteric diseases : Bacterial enteritis - dysbacteriosis

- The increasing prevalence of subclinical necrotic enteritis and dysbacteriosis are the most significant challenges to modern and antibiotic growth promoters free poultry production.
- The **mycotoxin deoxynivalenol predisposes for the development of necrotic enteritis in broilers.** Subclinical necrotic enteritis (NE) is an economically important enteric disease caused by Gram-positive, anaerobic bacterium, Clostridium perfringens. The Fusarium mycotoxin deoxynivalenol (DON) is a common feed contaminant and may damage intestinal epithelial cells and/or their intercellular junctions, subsequently inducing protein leakage (Girish and Smith, 2008) and may thus predispose to the development of NE.



4. Oxidative stress

Considering that mycotoxins are among the stress factors that have a negative effect on pro & antioxidant balance in the body and especially in the cell, reactive oxygen species can get out of balance. This may lead to a situation whereby **the bird is no longer able to quickly detoxify these products, leading to oxidative stress**.

Such a wide spectrum of challenges requires a **combined approach to maintain and improve the overall production condition**.

Solution via the drinking water

Animals suffering from disease disorders will reduce first feed intake while they maintain water consumption. Significant reduction in appetite and feed intake has severe consequence on the overall condition of the animal. Insufficient nutrient supply in general, and essential nutrient uptake in particular will quickly lead to an impaired overall condition. An irregular supply of nutrients to the intestinal tract will also influence negatively the micro-flora, intestinal integrity and overall animal performance.

Drinking water however offers a perfect medium for the application of supplements to overcome periods of stress, to recover faster from disease and to limit periods of reduced feed intake to a minimum. Even in case of urgency, there is no need to postpone a treatment because of feed reformulations or production as such treatment can be started immediately.

Application of additives via drinking water is a very practical and straight forward approach in farm management. The product and application can be set for 100% in function of the farmer's observation. He has finally the master's eye and is responsible to achieve the best performance and profitability.

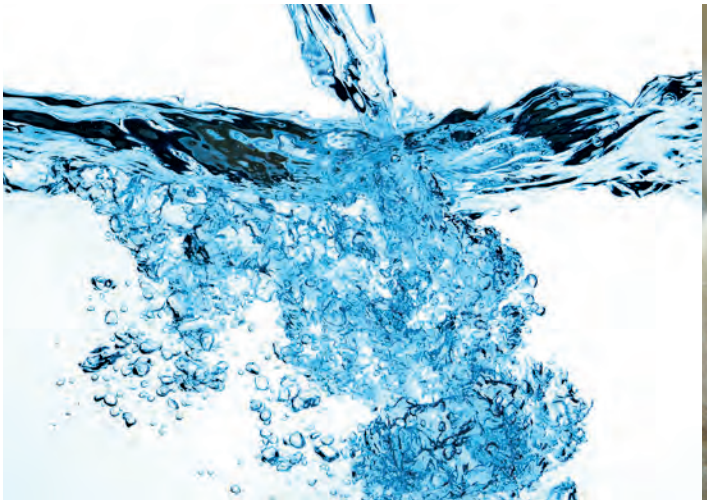
A drinking water treatment is a flexible approach in terms of timing and application.

Escent® is not available in the USA and Canada.

Escent® L

Specially designed product for drinking water application, which is synonym for flexibility, efficiency and profitability, and **a very wide action radius, ESCENT® L reduces & overcomes stress-induced health problems thanks to its :**

- **Diuretic effect**
- **Liver tonic, enhancing excretion of harmful metabolites**
- **Detoxifier power**
- **Immune response support**
- **Water intake improver**



ESCENT® L is an innovative liquid anti-stress, mycotoxins control and animal revitalization solution, combining **yeast extracts, Yeast fermentation products (Saccharomyces cervisiae) with bio-active contents**, chelators, energy sources, minerals, botanicals and organic acids. It ensures a critical supply of nutrients and supportive molecules to counteract the negative impact of feed refusal on metabolism and micro-flora. Botanicals will contribute to optimising metabolic processes while yeast extracts are known to support the animal's immune function.

ESCENT® L Bio-toxicosis Treatment & Prevention tool addresses toxins, endotoxins and hidden toxins challenges when feed treatment is too late or not practical.



ESCENT®L benefits:

- Provides **readily bio-available nutrients** (Vitamins, minerals, amino acids, energy)
- Provides excellent media for **beneficial bacterial growth**
- Vitalize damaged organs like **liver and kidneys**
- Maintains **lower pH** in the guts (acidification)
- Helps **exacerbating proper fermentation** and thus eliminates toxins of the guts mal-fermentation
- Detoxifies Mycotoxins by Biotransformation and Caption (wide spectrum & highly efficient in acid media)
- **Reduces immune suppression**
- Enhance animal **disease resistance** and defense system
- Reduces negative effect of **oxidative stress**
- **Reduces convalescence**
- **Improves overall performance** under healthy or diseased conditions in terms of FCR, homogeneity and production index

While being safe for the user, the animal, the equipment and the environment, **ESCENT® L** is non-corrosive, organic, bio-degradable, stable and water soluble suspension.

This liquid application is ideal in situations in which distressed animals reduce their feed intake but continue drinking.

Fig: Yeast fermentation products (Saccharomyces cervisiae) with bio-active contents

<table>
<tr><th colspan="2">Fermentation extract rich of Beta Glucan and Nucliotides</th></tr>
<tr><td>Fermentation Enzymes:
(Bio-transformers, pro-digestion)</td><td rowspan="2">Amino Acids:
Isoleucine, Valine, Methionine, Cystine, Phenylalanine, Tyrosine, Threonine</td></tr>
<tr><td>Vitamin:
B1, Vitamin B2, Vitamin B6, Vitamin B12, Biotine, PP</td></tr>
<tr><td colspan="2">Minerals: Potassium, Sulfur, Phosphorus, Magnesium</td></tr>
</table>

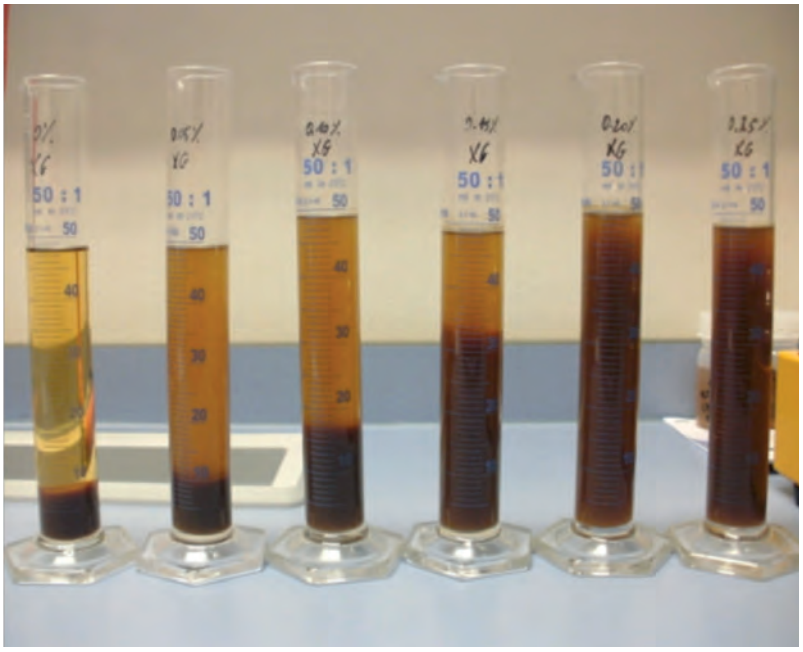
Escent® is not available in the USA and Canada.

Use & Application

Treatment can be started for all clinical cases of mycotoxicosis and the prevention thereof, as well as in circumstances of stress, severe disease conditions, immune-suppression or whenever animal performance is reduced.

CONDITION	DOSE	PERIOD
Under stress	1ml/l	Min 5 days
Mycotoxicosis treatment	1ml/l	Min 7 days
1st /last week of production	0,5 -1,0 ml/l	Min 5 days
Around vaccination	0,5 -1,0 ml/l	Min 5 days
Heat Stress	1 ml/l	Min 5 days

Packaging available (0,5 – 1 l)



Very stable liquid. No sedimentation.
ESCENT® L : 1st vial from the right

Escent® is not available in the USA and Canada.

Escent®L testimonials

Gulf countries

Under Clinical mycotoxicosis due to high ochratoxins contamination, high mortality, stunted growth, mouth lesions were observed prior to treatment with ESCENT® L:

Once ESCENT® L was added in the drinking water at a dose of 1ml per 1l of water, the following signs were recorded:

- More viable within 12 hours
- Water and feed consumption to normal within 24h
- Mortality reduced to normal within 48h

South East Asia

In the presence of intense heat stress, with analysed levels of endotoxins and mycotoxins, liver damages, feed intake was maintained regardless of the overall stress created.

Middle East

Selected poultry producers on trial with ESCENT® L noticed the following improvements when treating for a few days with ESCENT® L at 1ml/1l after normal disease treatment:

- Regain health parameters faster
- No new case development
- Recovery of organ activity
- Lower medication

Escent®L trials summary

Broilers

Trial size (# animals)	Observed abnormalities	Application	Observation
20 000	No specific issues	1 ml/l, day 2 - day 5	At 28 days, improved FCR and LW advanced for 2 days
28 000	Daily mortality higher then normal (0,625%)	1 ml/l, day 6 – day 11	Daily mortality 0,01%
60 000	No specific issues	20 ml/2000 heads for 4 days	Lower mortality, better FCR compared to historical data
175 000	Wet dropping during feed transitions (mash to crumble and crumble to pellet)	1 ml/l during 2-3 days	Improved manure quality, no wet droppings.

Layers/Breeders

Trial size (# animals)	Observed abnormalities	Application	Observation
20 000 layers	Egg prolapse	1 ml/l for 3 days	Significant improvement of egg prolapse
54 000 breeders	No specific issues	0,5 – 1 ml/l in growing breeders	Reduced mortality

Escent® is not available in the USA and Canada.

Product Data Sheet

ESCENT® L

Scope	Raw material, feed and drinking water quality accounts for the larger part of a successful intensive animal rearing operation. They require special care in order not to make them a source of mechanisms (poisonous substances, interference with digestibility, oxydation and free radicals) that affect feeding efficiency and animal production response. Such mechanisms can cause severe economic losses to the producer.
Description	Complementary feed. A well balanced liquid synergistic mixture of carefully selected additives for use in intensive livestock production.
Components	Plant extracts, Inactivated yeast and yeast extracts (Saccharomyces cerevisiae), Citric acid, Ortho-phosphoric acid, Lactic acid, Propylene Glycol, carrier.
Physical & Technical Specifications	**Appearance** : transparent to cloudy liquid **Colour** : light to dark brown **Density** : 1 - 1,2 kg/l **pH (10%)** : 1,5 - 2,5 **Colour change or variation does not affect performance.**
Application & Dosage	Feed: 1,0- 4,0 kg/T, Drinking Water:: 0,5 - 2,0 l/1000 l
Packaging	HDPE export worthy recipients on wooden fumigated pallets. Available net weights: (0,5 lt* / 1,0 lt* / 5 lt* / 25 kg /, 200 kg /, 1000 kg (IBC) / bulk. *Marked unit packs are packed in an outer carton up to max. 25 kg.
Shelf Life	2 years when stored in a cool and dry environment out of direct sunlight in unopened packing
Item Reference	**10037**

INNOV AD NV/SA
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2910 Essen - Belgium

PDS/2 DATED 19-12-2011

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