



**SÜD-CHEMIE**  
Creating Performance Technology

**TIXISORB®**

# **Mycotoxin Adsorbents for the Middle East Market**

Meeting with Gulf European General Trading L.L.C, Dubai,

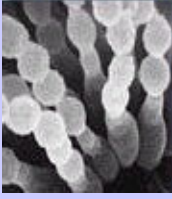




in October 2006

Peter-C. Schillok, agrimont GmbH, Germany

# Most Important Moulds and Their Toxins



**SÜD-CHEMIE**  
Creating Performance Technology

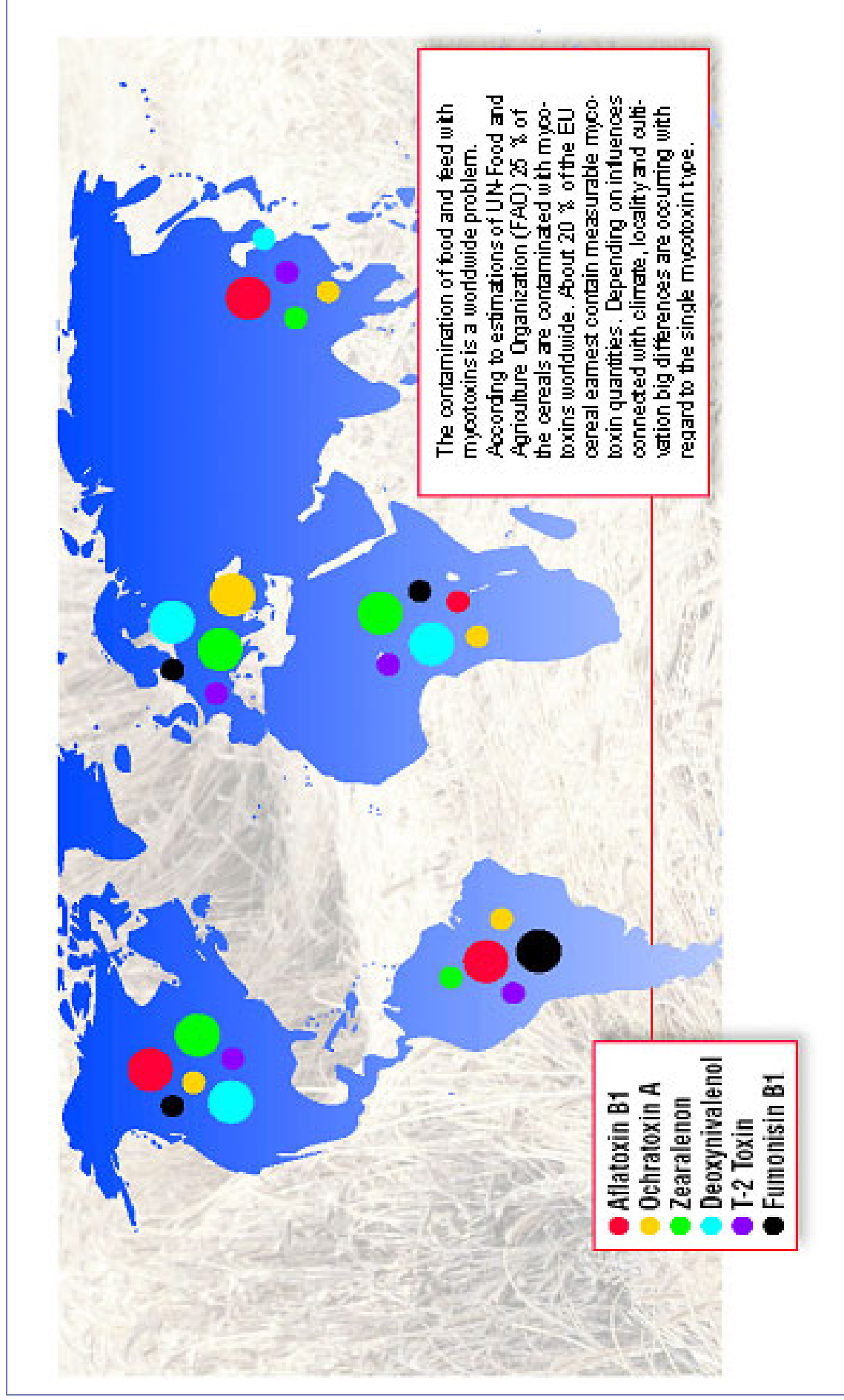
				
<b>Aspergillus*</b>	<b>Penicillium*</b>	<b>Fusarium</b>	<b>Alternaria</b>	<b>Claviceps</b>
<ul style="list-style-type: none"> <li>• <u>Aflatoxin B1, G1, M1,</u></li> <li>• <u>Ochratoxin A</u></li> <li>• <u>Sterigmatocystin</u></li> <li>• <u>Cyclopiazonic acid</u></li> </ul>	<ul style="list-style-type: none"> <li>• <u>Ochratoxin A</u></li> <li>• <u>Citrinin</u></li> <li>• <u>Patulin</u></li> <li>• <u>Cyclopiazonic acid</u></li> </ul>	<ul style="list-style-type: none"> <li>• <u>Trichothecenes (Deoxynivalenol, Nivalenol, T2-Toxin, HT2-Toxin, Diacetoxyscirpenol)</u></li> <li>• <u>Zearalenone</u></li> <li>• <u>Fumonisin B1, B2, B3</u></li> <li>• <u>Moniliformin</u></li> </ul>	<ul style="list-style-type: none"> <li>• <u>Tenuazonic acid</u></li> <li>• <u>Alternariol</u></li> </ul>	<ul style="list-style-type: none"> <li>• <u>Ergot alkaloids</u></li> </ul>

\* storage fungi

# Global Occurrence of Main Mycotoxins



**SÜD-CHEMIE**  
Creating Performance Technology



# Regional Relevance of Mycotoxins



**SÜD-CHEMIE**  
Creating Performance Technology

	AFB1	OTA	FB1	DON	T-2	ZON
North America	+	+	+	++	0	++
Mexico / Central Am.	++	+	++	0	++	+
South America	++	0	++	+	++	+
EU 15	-	+	+	++	0	++
EU East / Middle East	+ / ++	+	++	+	0	+
Afrika	++	0	++	0	+	+
Asia / Pacific	++	0	++	0	++	+
Russia	+	+	+	++	+	++
Australia	+	0	+	0	0	+

Legend: ++ highly relevant

+ relevant

0 occurring

- low relevance

-- no relevance

# Effects of Mycotoxins to Farm Animals



**SÜD-CHEMIE**  
Creating Performance Technology

	NOEL* (No observable effect level) [ppb]	Effects / Symptoms in the field
<b>Aflatoxin B1</b> (AFB1)	pig 5 - 20 poultry 5 - 20 cattle 5 - 20	Decreased feed uptake & feed efficiency, suppression of immune system / lower resistance to diseases, reduced weight gain, reduced milk production, declined reproductivity
<b>Ochratoxin A</b> (OTA)	pig 20 - 100 poultry 100 - 200 cattle 200 - 1000	Suppression of immune system disordered kidney function, damage of kidneys decreased egg production
<b>Ergot Alkaloids</b>	pig < 0.1% sclerotia poultry < 0.1% sclerotia cattle < 0.1% sclerotia	Decreased feed consumption & feed efficiency, suppression of immune system, cramps, necrosis of ears and tails, milk reduction, abortion
<b>Fumonisin B1</b> (FB1)	pig 1000 - 2000 poultry 1000 - 2000 cattle 2000 - 4000	Decreased feed consumption, slower growth, damage of liver and lungs, pulmonary edema (pigs), brain damage (horse)

\* References: Prof. Dr. J. Bauer, Technical University of Munich, 1998  
Prof. Dr. J. Fink-Gremmels, University of Utrecht, 1999  
Dr. C. R. Hurburgh, Iowa State University, 1996

# Effects of Mycotoxins to Farm Animals



Mycotoxin	NOEL* (No observable effect level) [ppb]	Effects / Symptoms in the field
<b>Deoxynivalenol</b> (DON, Vomitoxin)	pig 100 - 200 poultry 1000 - 2000 cattle 100 - 200	feed refusal, vomiting, decreased weight gain, suppression of immune system
<b>Zearalenone</b> (ZON)	pig 20 - 50 poultry 500 - 1000 cattle 100 - 500	decreased fertility (oestrogenic response), disordered reproduction decreased feed consumption, declined growth
<b>T-2 Toxin</b>	pig 200 - 1000 ppb poultry 100 - 200 ppb cattle 200 - 1000 ppb	decreased feed uptake, slower growth, drop in egg production, suppression of immune system, lesions, sterility

\* References: Prof. Dr. J. Bauer, Technical University of Munich, 1998  
 Prof. Dr. J. Fink-Gremmels, University of Utrecht, 1999  
 Dr. C. R. Hurburgh, Iowa State University, 1996

## **Possibilities to Protect Farm Animals from Damages Caused by Mycotoxins:**

- physical chemical methods  
➔ low efficiency, high costs
- adsorptive binding to prevent resorption of toxins from the digestive tract

➔ Various products on the market already, mainly based on aluminosilicate minerals like bentonite, kaolinite, zeolithe and sometimes organic compounds.

## Range of **TOXISORB**<sup>®</sup>

Products offered

by SÜED – CHEMIE:

### **FIXAT**

for Aflatoxins + Ergot Alcaloides

### **TOXISORB Plus**

for Aflatoxins + Ergot Alcaloides

(optionally)

+ Fumonisin + CPA + T-2 Toxin

### **TOXISORB Premium**

for Aflatoxins + Ergot Alcaloides

+ Fumonisin + CPA + T-2 Toxin

+ Ochratoxin A + DON / Vomitoxin

+ Zearalenone

# Mineral Adsorbent Based on Bentonite - Montmorillonite



**SÜD-CHEMIE**  
Creating Performance Technology

**Al-Silicates** → any mineral containing Al + SiOH

→ amorphous / no crystalline structure

→ 3-dimensional structure (e.g. Zeolites)

→ layered silicate minerals = **Phyllosilicates**

- Kaolinite: 2-layered
- **Smectite** / Illite: 3-layered
- Sepiolite / Palygorskite: 3-layered + linked

**Smectites:** - Nontronite (rich in Fe)

- Beidellite (rich in Al)

- **Montmorillonite** (rich in Mg)

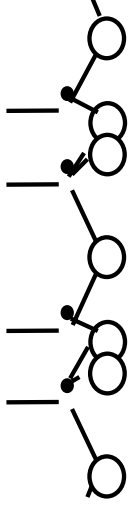
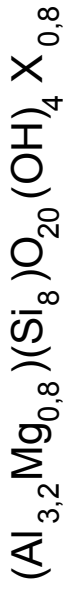
**Bentonite:** Rock, containing mainly Montmorillonite plus accompanying minerals

# Crystal Structure



**SÜD-CHEMIE**  
Creating Performance Technology

## Montmorillonite:



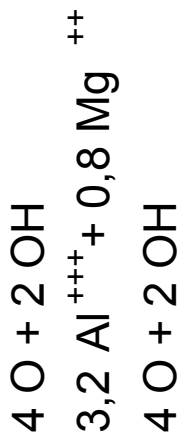
## „INTERLAYER“



## TETRAHEDRON



## OCTAHEDRON



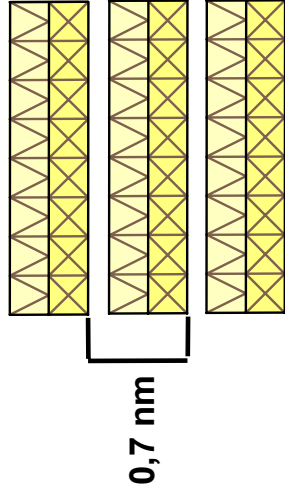
## TETRAHEDRON



# Mineral Adsorbent Based on Bentonite - Montmorillonite

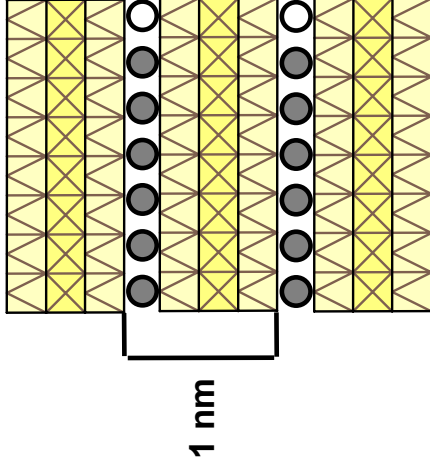
## two layered -

e.g. **KAOLINITE**

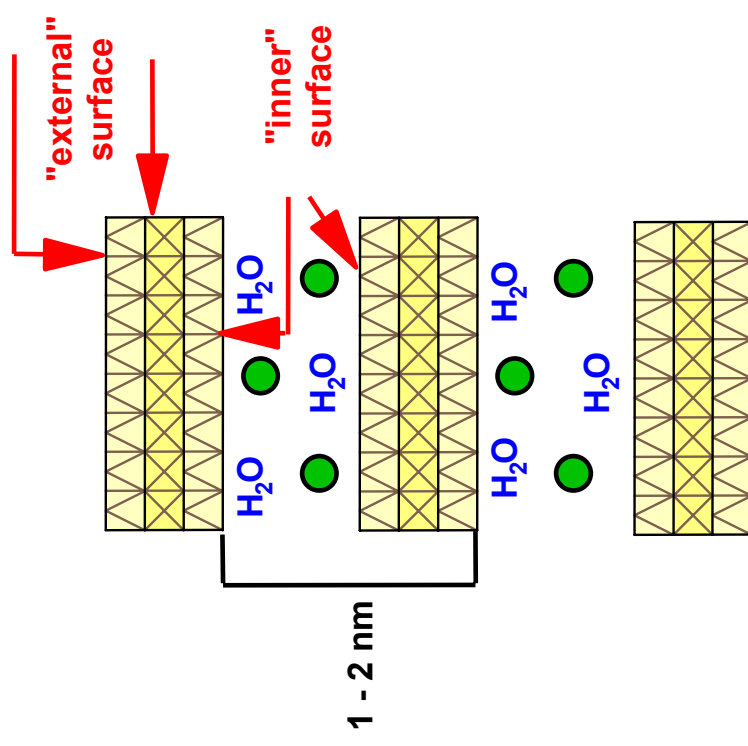


## three layered - clay minerals

e.g. **ILLITE**



e.g. **MONTMORILLONITE**



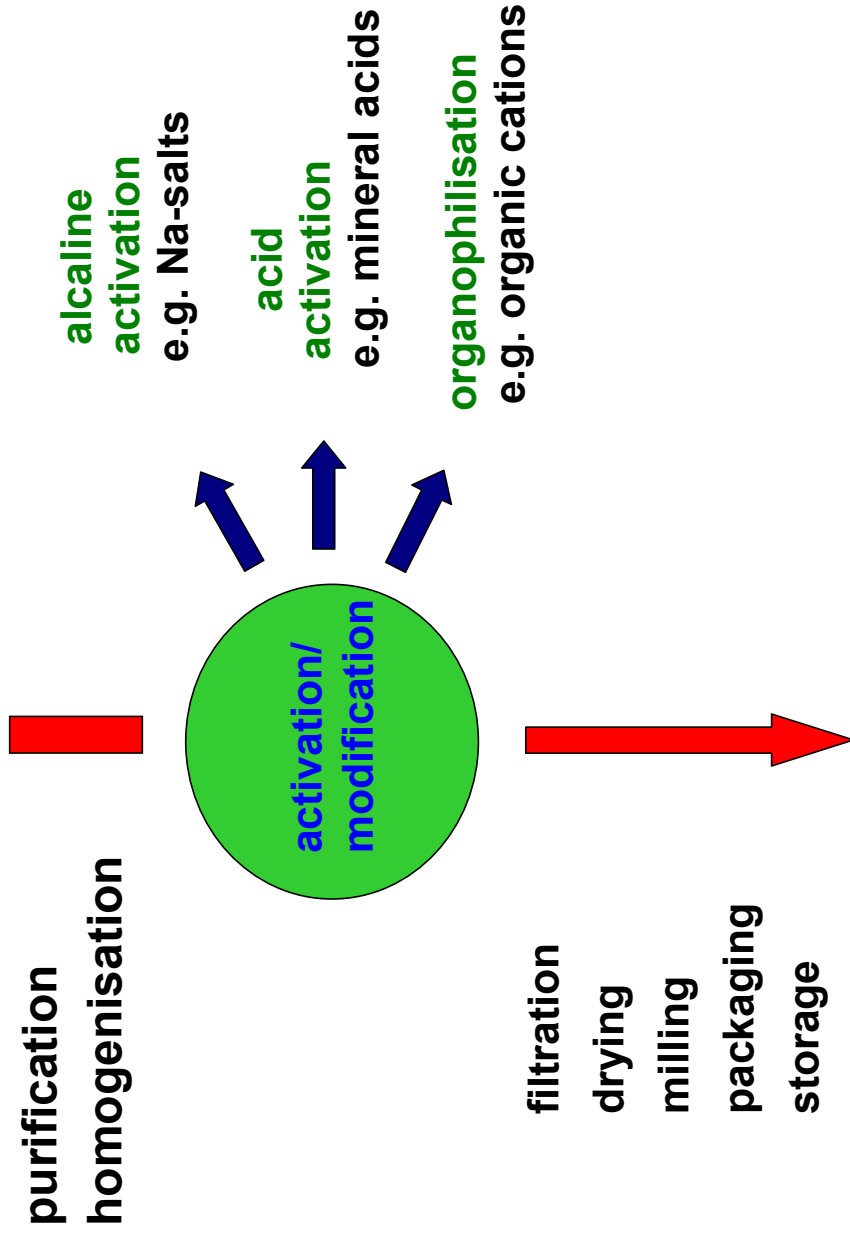
- fixed K - ions
- exchangeable potassium
- exchangeable Ca-, Mg-, Na-ions

# TOXISORB Production Process



**SÜD-CHEMIE**  
Creating Performance Technology

## Raw-Bentonite



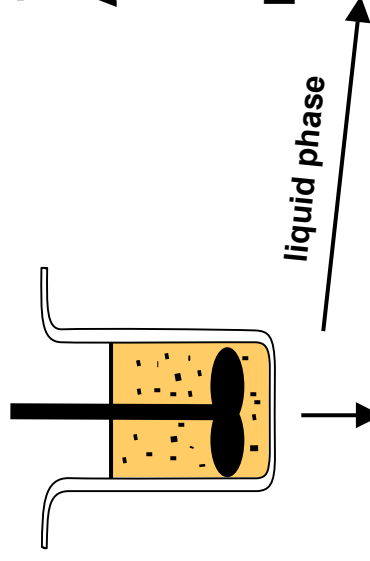
**TOXISORB**<sup>®</sup>

## 1. step: Adsorption Under Acidic pH-Conditions

A) aqueous toxin solution, buffered at pH 3 (mimicking stomach pH) + adsorbent (0.2 – 0.4 % [w/w]), agitating 2 h at 37 °C

B) centrifuging (10 min 2800 U / min)

- clear supernatant to HPLC for analysis of remaining toxin amount
- remaining solid phase is used in a desorption step

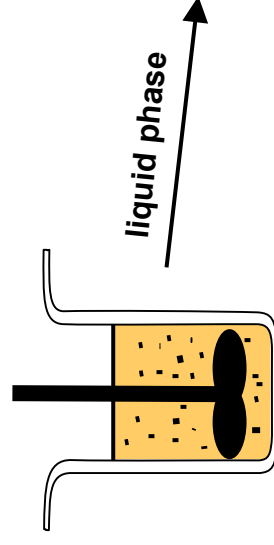


## 2. step: Desorption Under Neutral pH-Conditions

C) resuspension of solid phase (= adsorbent + bound toxin) in aqueous solution at neutral pH (mimicking intestine), agitating 2 h at 37 °C

D) centrifuging (10 min 2800 U / min)

- clear supernatant to HPLC for analysis of desorbed toxin amount

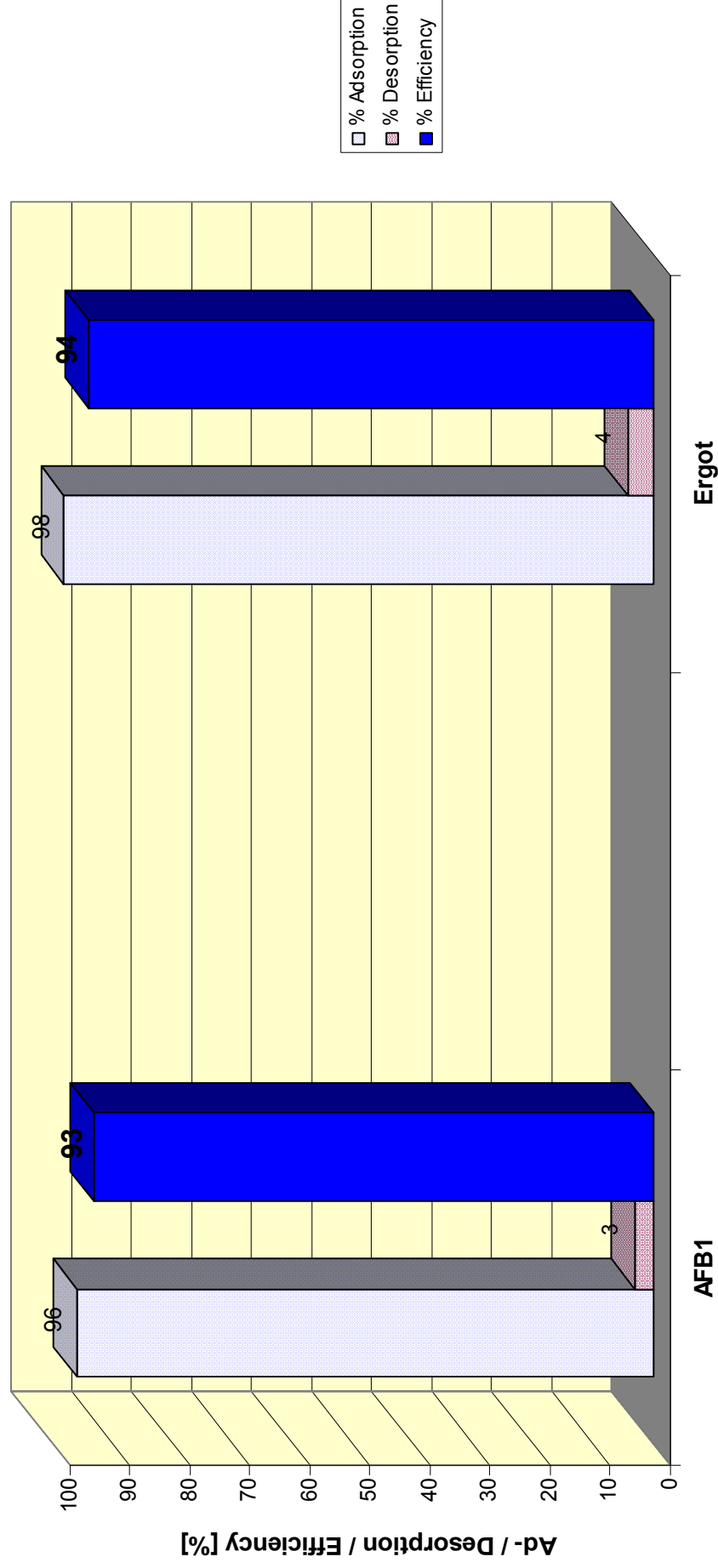


**Efficiency = % Adsorption - % Desorption**

## In-Vitro Results of FIXAT

Toxin conc.: AFB1 : 2000ppb ; Ergotamin : 1500ppb

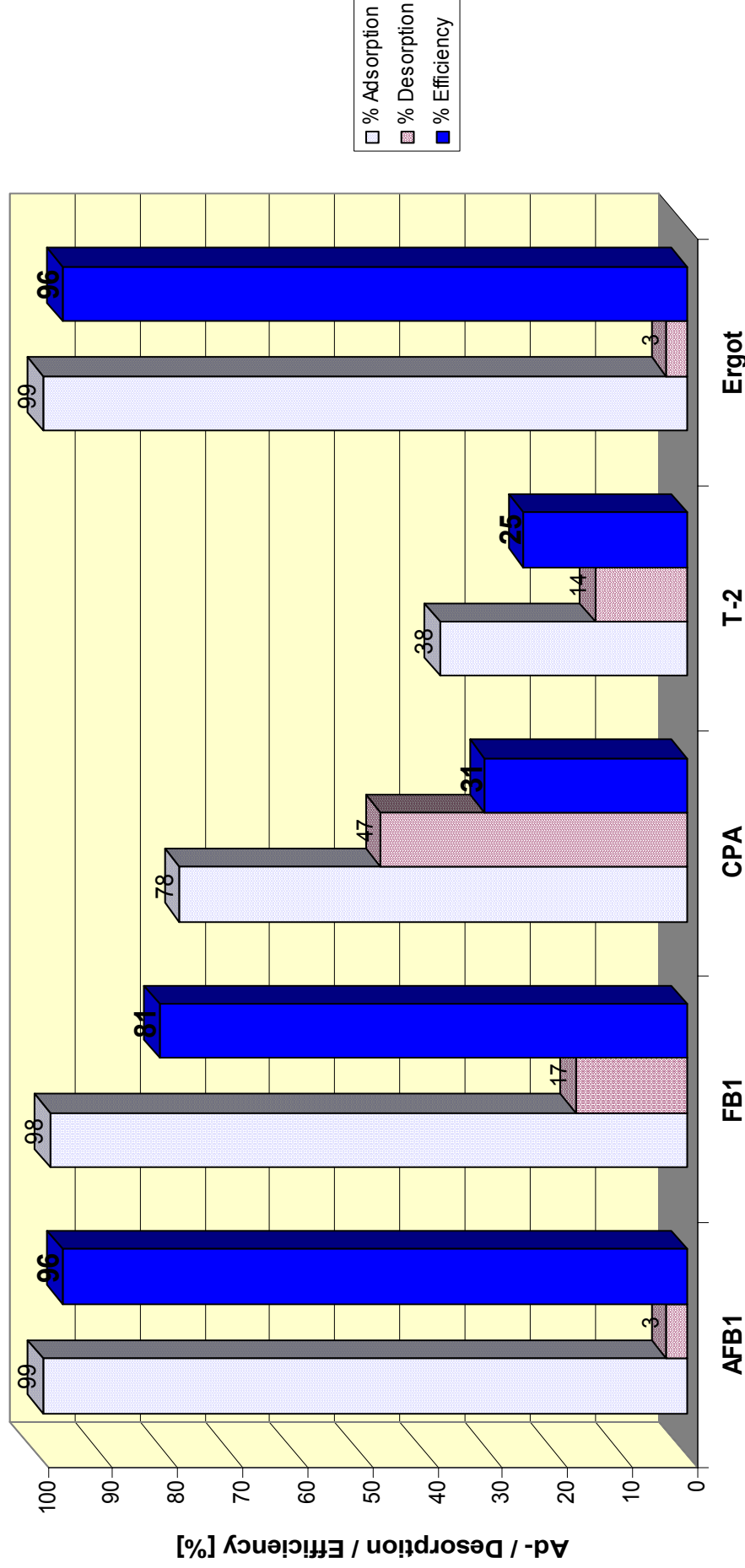
Dosage of adsorbent: 0,4%; pH adsorption 3,0 ; pH desorption 6,5



## In-Vitro Results of TOXISORB Plus

Toxin conc.: AFB1, CPA, T-2 : 300ppb ; FB1, Ergotamin : 1500ppb

Dosage of adsorbent: 0,4%; pH adsorption 3,0 (T-2: 4,5) ; pH desorption 6,5



# TOXISORB Premium

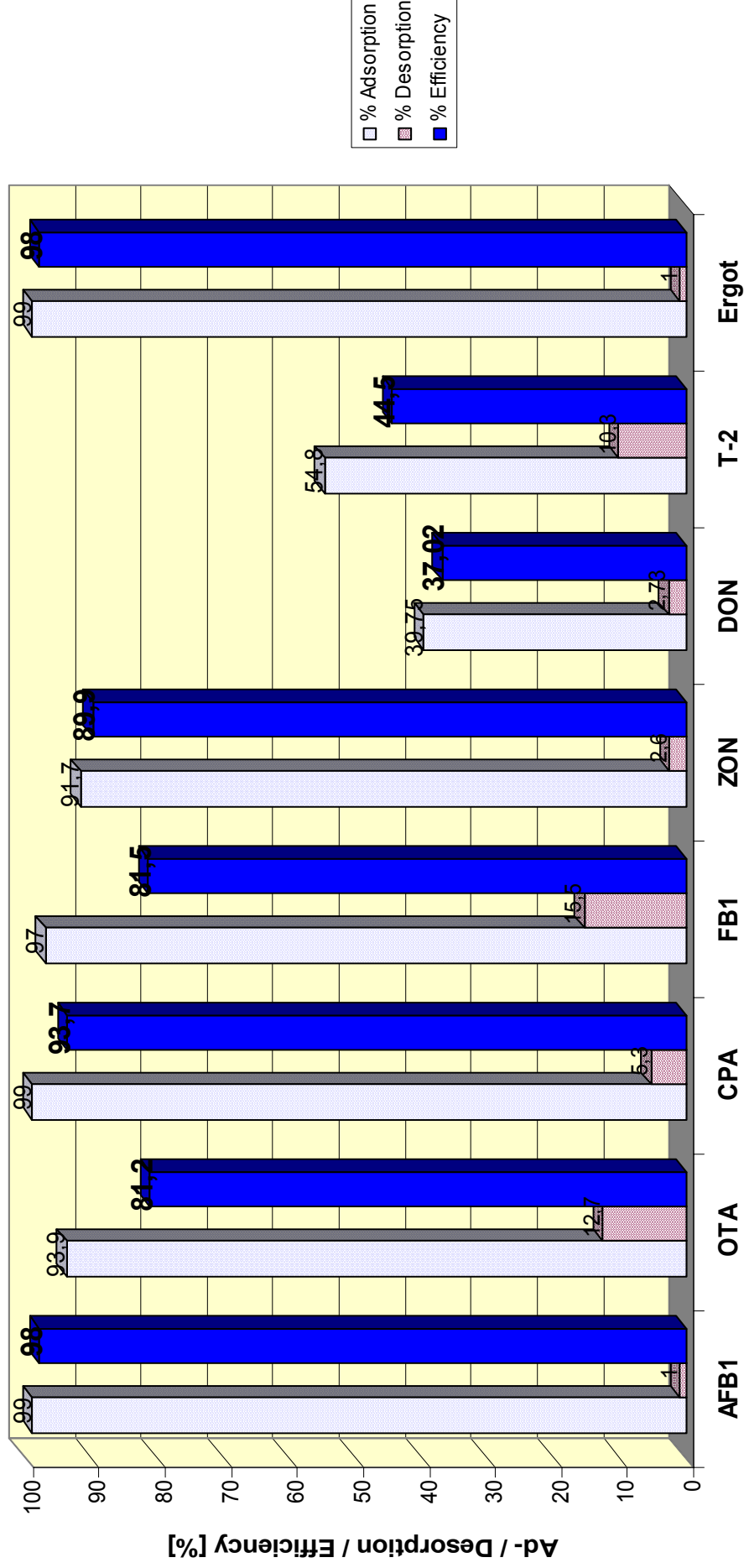


**SÜD-CHEMIE**  
Creating Performance Technology

## In-Vitro Results of TOXISORB Premium

Toxin conc.: AFB1, CPA, T-2 : 300ppb ; FB1, Ergotamin : 1500ppb

Dosage of adsorbent: 0,4%; pH adsorption 3,0 (T-2: 4,5) ; pH desorption 6,5



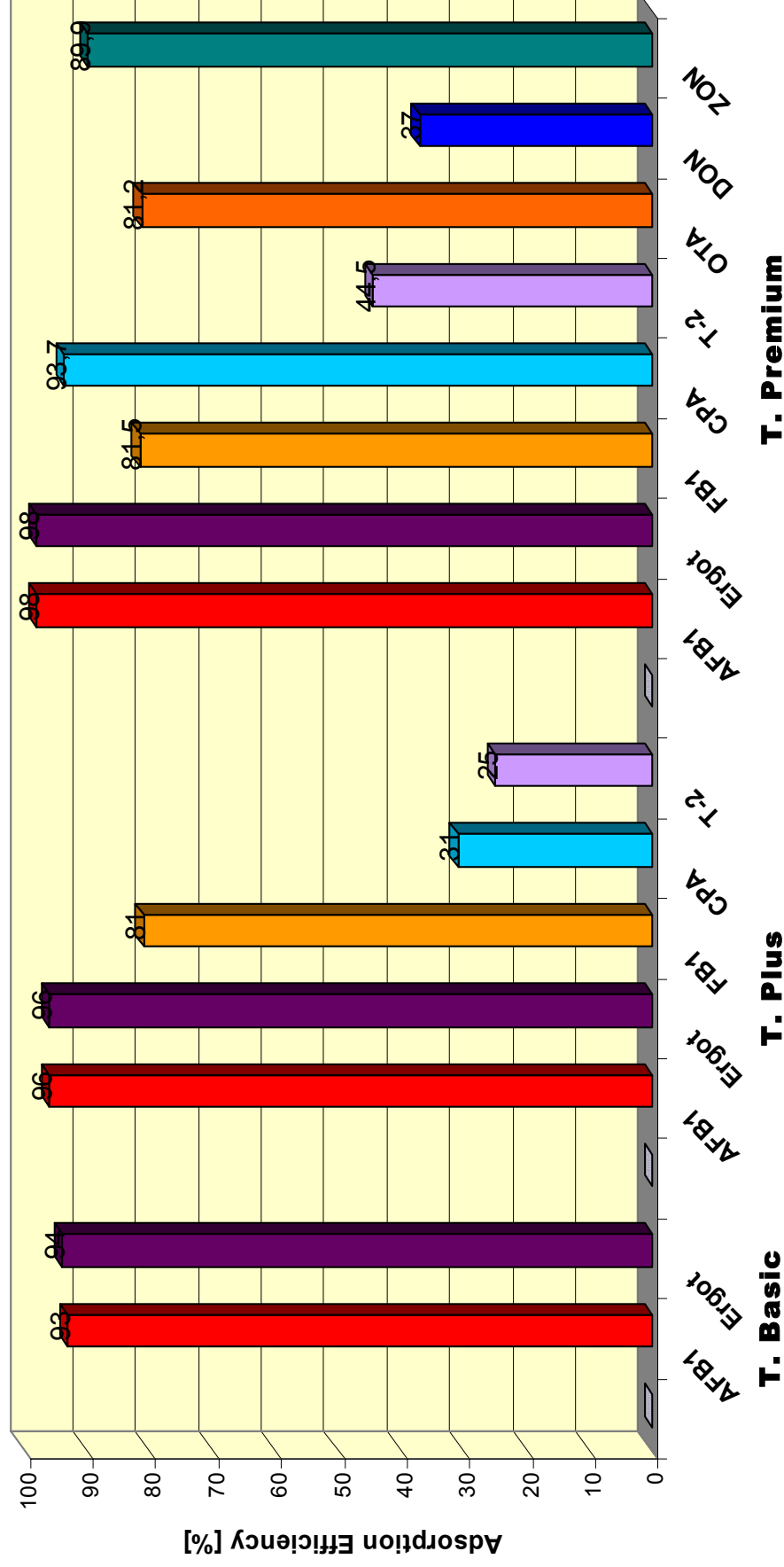
# TOXISORB Product Range



**SÜD-CHEMIE**  
Creating Performance Technology

## In-Vitro Results of TOXISORB Product range

Toxin conc.: AFB1, OTA, CPA, ZON, T-2 : 300ppb ; FB1, Ergotamin, DON : 1500ppb  
 Dosage of adsorbent: 0,4% ; pH adsorption 3,0 (T-2: 4,5) ; pH desorption 6,5



# Sensitivity of Farm Animals



**SÜD-CHEMIE**  
Creating Performance Technology

Mycotoxin	Pig	Poultry	Ruminants
Aflatoxin B	+++++	+++++	+++++
Ochratoxin A	+++	++	+
Ergot	+++	++	+++
Fumonisin	+++	++	+*
Deoxynivalenol	+++	+	+
Zearalenone	+++++	+	++
T-2 Toxin	++	++++	++

sensitivity: + = low, ++ = medium, +++ = high, ++++ = very high

\* horses are high sensitive

# Relevance of TOXISORB Qualities



**SÜD-CHEMIE**  
Creating Performance Technology

	AFB1	OTA	FB1	DON	T-2	ZON		T. Basic	T. Plus	T. Premium
North America	+	+	+	++	0	++	↑	+	++	++
Mexico / Central Am.	++	+	++	0	++	+	↑	++	++	++
South America	++	0	++	+	++	+	↑	++	++	++
EU 15	-	+	+	++	0	++	↑	-	+	++
EU East / Middle East	+ / ++	+	++	+	0	+	↑	++	++	++
Afrika	++	0	++	0	+	+	↑	++	++	+
Asia / Pacific	++	0	++	0	++	+	↑	++	++	+
Russia	+	+	+	++	+	++	↑	+	++	++
Australia	+	0	+	0	0	+	↑	+	+	+

Legend: ++ highly relevant

+ relevant

0 occurring

- low relevance

-- no relevance



## ***In-vivo* Results**

- from scientific trials:
  - La Molina, Peru
  - INIFAP, Mexico
  - Monterrey, Mexico
  - Santa Maria, Brasil
  
- from field tests:
  - Peru,
  - Mexico,
  - Brasil,
  - Germany



## **FIXAT® - In-Vivo Test with Broiler Chicken**

**at Universidad Agraria La Molina, Lima, Peru**

Feeding of day old male broiler chicken with a diet contaminated with 2500 ppb Aflatoxin B1 over 28 days

Variants	added amount of FIXAT	Aflatoxin B1	dressed weight [g]	feed consumption [g]
Control FIXAT	- 0.50%	- -	1235 (a) 1207 (a)	1708 (a) 1704 (a)
Control FIXAT	- 0.50%	2.5 ppm 2.5 ppm	1150 (b) 1218 (a)	1600 (b) 1695 (a)

The treatments consisted of three repetitions with five heads each

### **Conclusion:**

# FIXAT added with 0.5% to the contaminated diet is able to neutralize the negative effect of 2.5 ppm Aflatoxin B1 completely.

# FIXAT shows no negative effect to animal growth (e.g. due to fixation of vitamins or trace elements).



## **FIXAT® - In-Vivo Test with Mice at INIFAP, Mexico**

Feeding of mice with a diet contaminated with 400ppb Aflatoxin B1 over 6 weeks

Treatment		Weight gain after 6 weeks (g)	Calcium [mg /dl blood serum]	Magnesium	Vitamin A (A1 + A2) [mg/kg liver tissue]	Vitamin B (B1, B2, B6)
AFB1 (ppb)	FIXAT (%)					
1	0	11.3	10.05	2.93	3.5	79.5
2	400	-1.8				
3	400	7.6				
4	400	9.6	10.21	2.85	3.53	78.4

**Conclusion:** # FIXAT® in a dosage of 0.5% (= 5 kg / mt) was able to compensate the effect of 400 ppb AFB1 by 67 %, in a dosage of 1.0 % by 85 %.

# Up to a dosage of 1.0% FIXAT® does neither influence the status of trace elements in blood serum nor that of Vitamin content in liver tissue.



**SÜD-CHEMIE**  
Creating Performance Technology

# **TOXISORB<sup>®</sup> - Feeding Trial with Broiler Chicken**

Reduction of Ochratoxin A residues in liver & effect to femur  
(University of Monterrey, Mexico, July 2000)

Treatment	OTA in liver [µg/kg]	Femur ash [%]	Femur resistance [N/m <sup>2</sup> ]
control without toxin	0 <sup>a</sup>	20.45	27557 <sup>a</sup>
control without toxin + TOXISORB (0.5% / 1.0%)	0 <sup>a</sup>	21.07	27789 <sup>a</sup>
diet with OTA (500ppb)	84 <sup>b</sup>	20.91	21167 <sup>a</sup>
diet with OTA (500ppb) + TOXISORB (0.25% / 0.5%)	36 <sup>c</sup>	21.06	26336 <sup>a</sup>
diet with OTA (500ppb) + TOXISORB (0.5% / 1.0%)	16 <sup>d</sup>	20.06	27168 <sup>a</sup>

Male broilers, 5 treatments (4 repetitions á 12 animals), trial period 6 weeks



# **TOXISORB®** - Feeding Test with Piglets

	<b>control group</b> contaminated feed without TOXISORB	<b>TOXISORB group</b> contaminated feed +0.6 % TOXISORB
number of animals	59	60
losses	0	2
weight at beginning	405	425
weight/animal	6.86	7.08
weight after 18 days	605	650
weight/animal	10.25	11.2
weight gain/animal	3.39	4.1
daily weight gain/animal	188	228
feed consumption/animal/d	333	354
feed conversion	1.78	1.55

**Results:** The TOXISORB® group shows a **21 % higher weight gain** than the control group. This is due to a **6.3 % higher feed consumption** in combination with an **increased feed conversion**. The piglets of the control group showed a depressed development in the first week as well as a rough appearance. In contrary to that the piglets of the TOXISORB® group accepted the diet well, showed a vital behavior from the beginning and developed good muscles.

The feeding test was done at the Schweineaufzuchtstation of WF Aufzucht GmbH, Freyenstein, Germany, in 2000.

# **TOXISORB® - Feeding Test with Piglets**

Effects of TOXISORB® on Trace Elements and Vitamin Status after 2 Weeks' Testing

Blood Serum Amounts of:	Control without TOXISORB	A + 0.4 % TOXISORB	B + 0.6 % TOXISORB	(Normal Values)
Calcium [mmol/l]	2.62	2.56	2.5	(2.0 – 3.0)
Magnesium [mmol/l]	1.75	1.39	1.67	(0.7 – 1.6)
Iron [µg/dl]	149	150	127	(93 – 107)
Copper [µg/dl]	183	194	138	(80 – 150)
Zinc [µg/l]	1119	811	1059	(700 – 1500)
Vitamin A [mg/l]	0.29	0.27	0.35	(0.2 – 1.2)
Vitamin E [mg/l]	0.7	1.4	1.2	(2.8 – 7.4)

Analysis done by: Institute for Clinical Testing Ludwigsburg GmbH, Laboratory for Veterinary Medicine

## **Results:**

**TOXISORB®** did not significantly influence the amount of important trace elements and vitamins in the bloodserum of piglets after 2 weeks of feeding 4 kg respectively 6 kg / to of feed.

# FIXAT® - In-Vivo Test with Broiler at a Peruvian Farm

Feeding of broiler with a diet slightly contaminated with Aflatoxin and T-2 Toxin over 45 days

parameters	control group (contaminated feed)	FIXAT group (contaminated feed + 0,25 % Fixat)
final body weight (kg)	2.457	2.599
weight of carcass (kg)	1.731	1.834
daily weight gain (g)	53.7	56.9
feed consumption (kg)	4.880	4.790
feed conversion	1.99	1.84
mortality (%)	5.11	5.97
<b>Productive Efficiency Index</b>	<b>260</b>	<b>295</b>

Each group consisted of 10.000 broiler, 50 % male / 50 % female

**Conclusion: FIXAT® in a dosage of 0.25 % (=2.5 kg / mt) was able to**

- # increase final body weight by 5.8 %
- # increase waight of carcasse by 6.0 %
- # improve feed conversion by 7.5 %
- # improve Productive Efficiency Index by 13.5 %