Collateral damages of the herbicide glyphosate in dairy cows, current possibilities to neutralize this contamination

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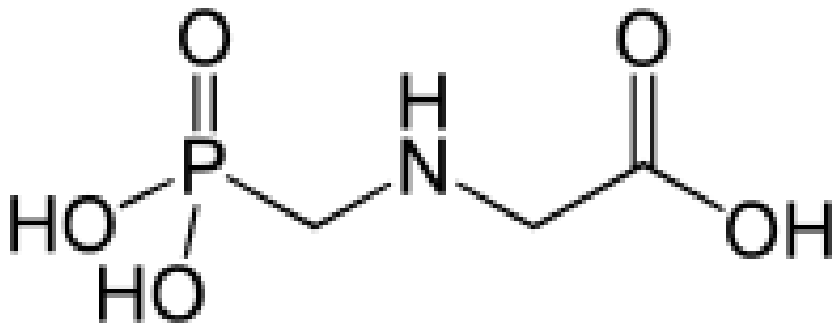
Berlin, 10.09-11.09. 2014

Bane and blessing of agrochemicals in industrialized agriculture and their backlash of humans, animals, and nature

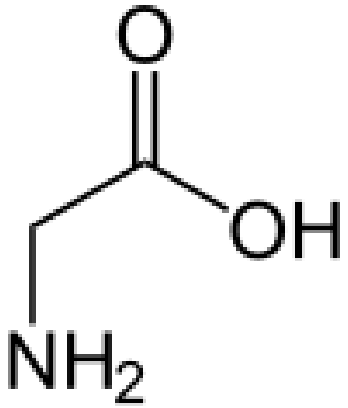
Umweltinstitut München, 2012

BUND 2012

Glyphosate



***N*-(Phosphonomethyl)-glycine**



Glycine

Glyphosate -Activities

- **Chelator** (Martin, 1950)
- **Herbicide** (admitted in USA 1974)
- **Bacteriostatic activity** (Abraham et al. 2010)
- **Cytotoxic activity** (Camden et al. 1996)

More glyphosate activities

(Samsel and Seneff, 2013)

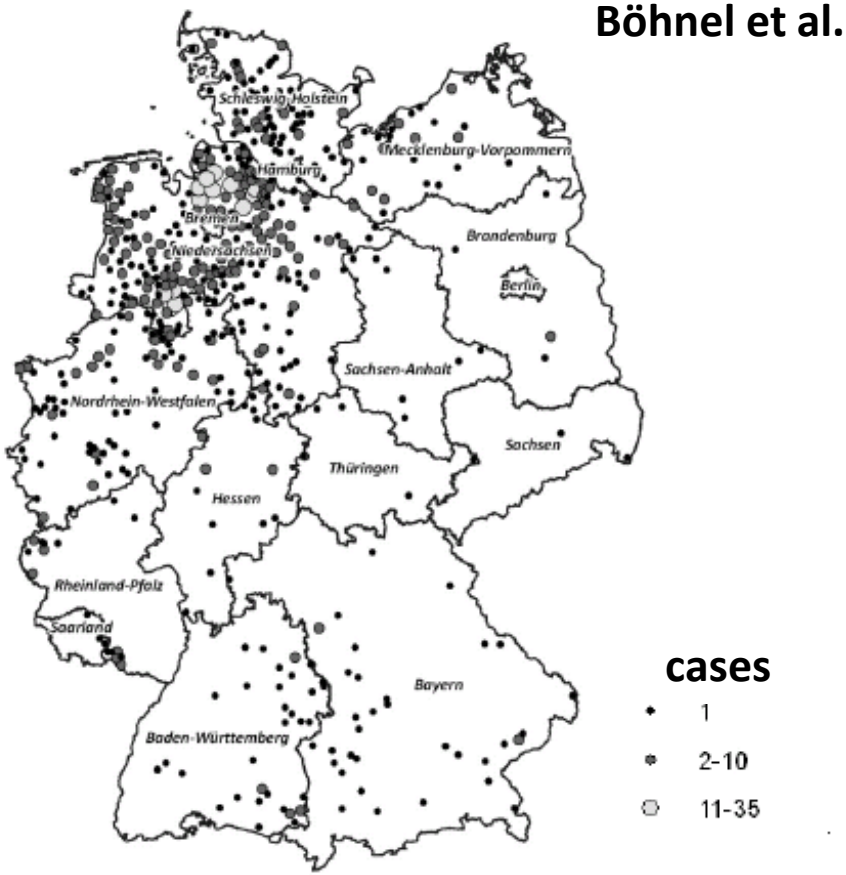
- **Inhibition of cytochrome P450 (CYP) enzymes**
- **Reduced detoxification of xenobiotics**
- **Slowly manifestation of inflammation damages throughout the body**
- **Hormon disruptor**
- **Disruption of biosynthesis of aromatic amino acids and related substances by gut bacteria**
- **Intravascular disseminated coagulation due to "salting out" of blood proteins**

Disseminated intravascular coagulopathy (Gerlach, 2012)

Reasons of interests in glyphosate

- **From the middle of 1990s unexplainable increased cases of chronic or visceral botulism**

Böhnel et al. 2011



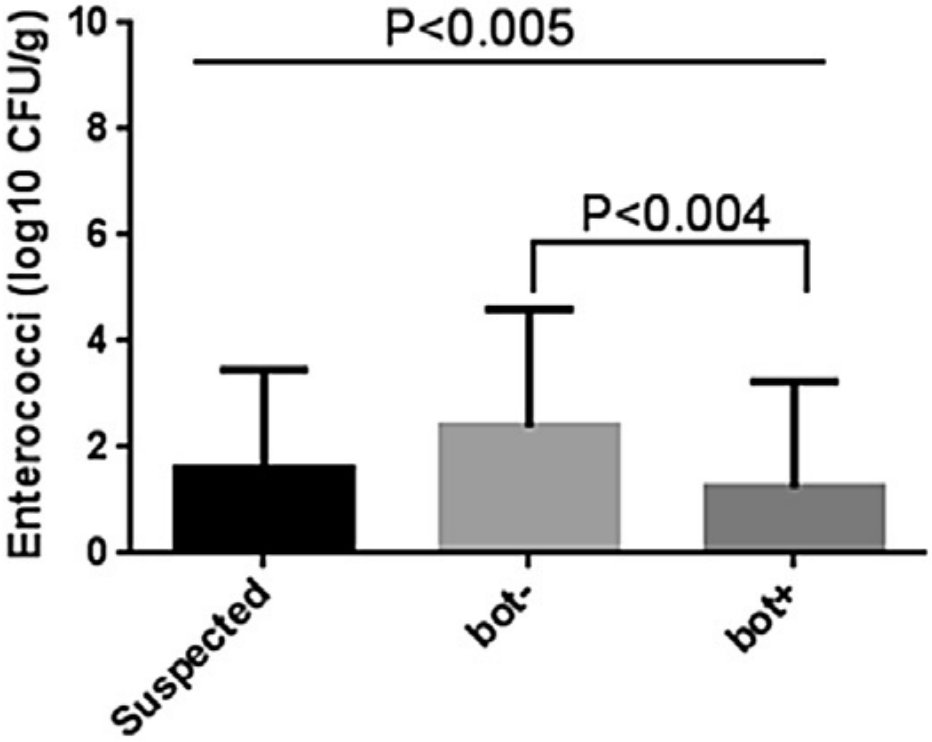
What are the reasons of chronic botulism?

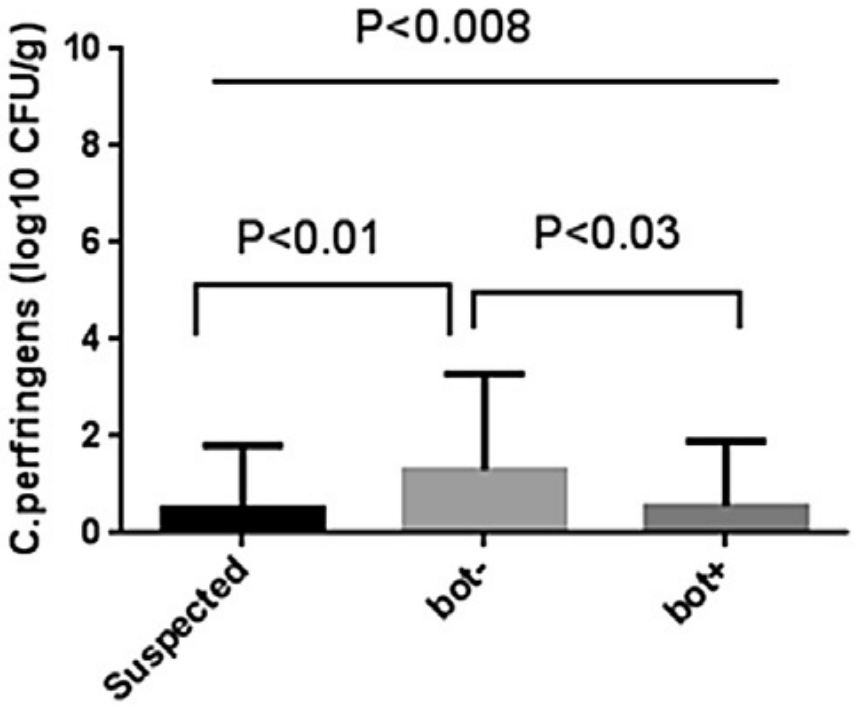
- **Most clostridiosis are opportunistic or factor dependent diseases**

- **In cattle *C. botulinum* type C and D intoxi-cations and toxicoinfections predominate, last one is a new disease in Germany, not approved by governmental authorities, but with growing importance and nevertheless the cows die !!**

Relationship between gastrointestinal dysbiosis and *Clostridium botulinum* in dairy cows (2007-2010)

M. Krüger et al. / Anaerobe 27 (2014) 100-105

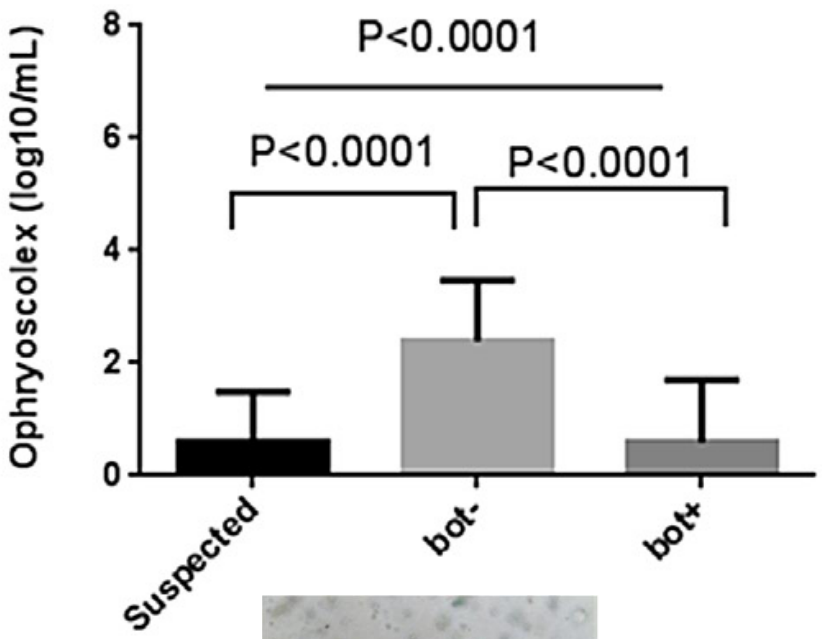


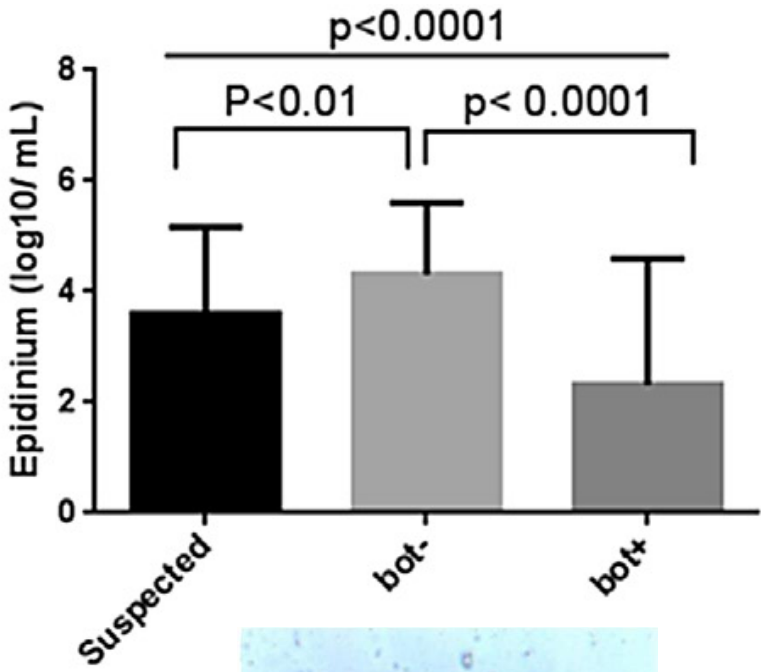


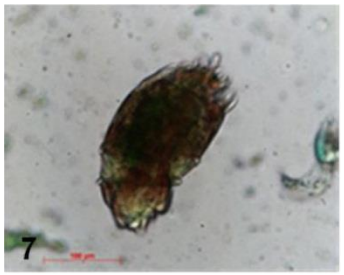
Bacteria in feces

Relationship between gastrointestinal dysbiosis and *Clostridium botulinum* in dairy cows (2007-2010)

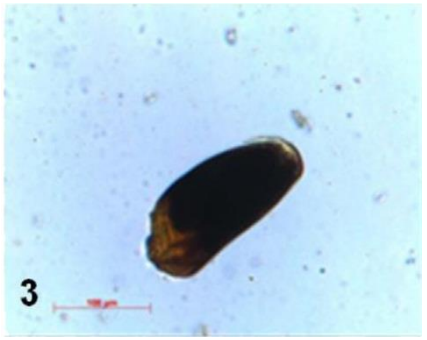
M. Krüger et al. / Anaerobe 27 (2014) 100-105







Protozoa in rumen fluids



In search for reasons of chronic botulism

- In 2010 we got the information about the herbicide glyphosate used in Germany for soil treatment and preharvest siccation as well as contamination of GMO soy admitted for feed and food in EU
- Two patents

US 7,771,736 B2
Aug. 10, 2010

EP 2 327 785 A2
01.06.2011

about antimicrobial activities of glyphosate influenced the next steps of investigations

Bacteria exhibit two forms of 5-enolpyruvyl-shikimat-3-phosphat (EPSP) synthases

- **Class I: sensible for glyphosate in micromolar concentrations**
- **Class II: still active in presence of glyphosate**

Sensible

Lactobacillus spp.
Enterococcus spp.
Bifidobacterium spp.
Bacillus spp.

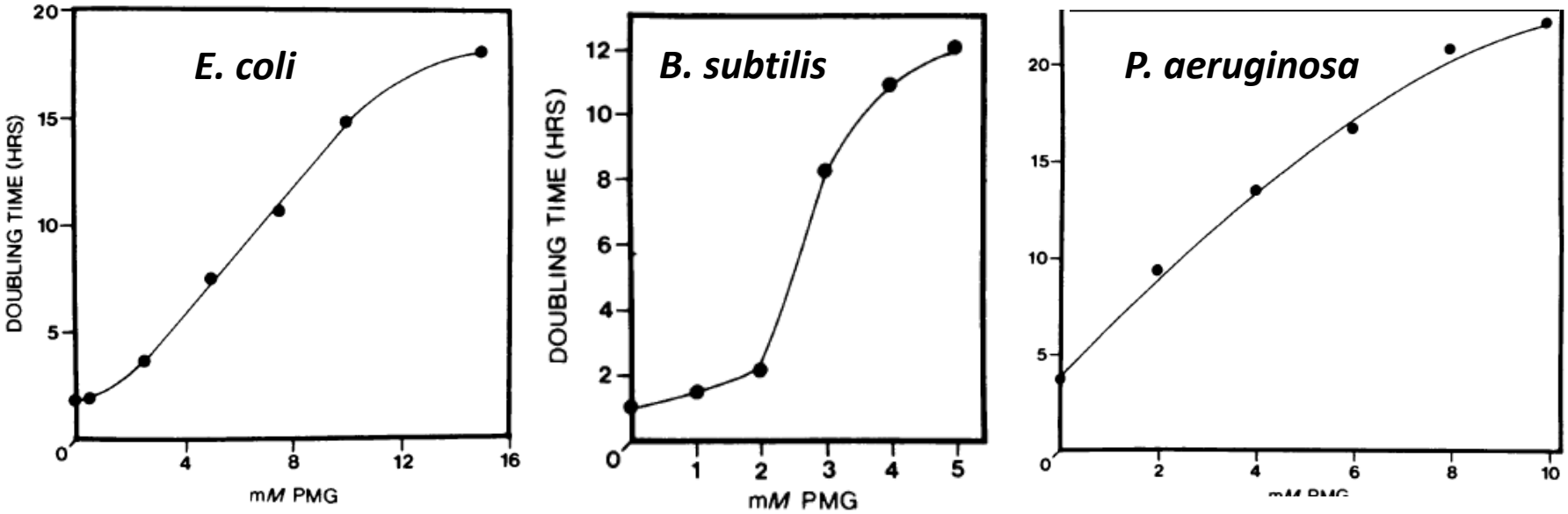
Resistent

Salmonella Typhimurium
Salmonella Enteritidis
Salmonella Gallinarum
Clostridium tetani
Clostridium perfringens
Clostridium botulinum

Shehata et al. 2012 **The Effect of Glyphosate on Potential Pathogens and Beneficial Members of Poultry Microbiota In Vitro**

Krüger et al. 2013 Glyphosate suppresses the antagonistic effect of *Enterococcus* spp. on *Clostridium botulinum*

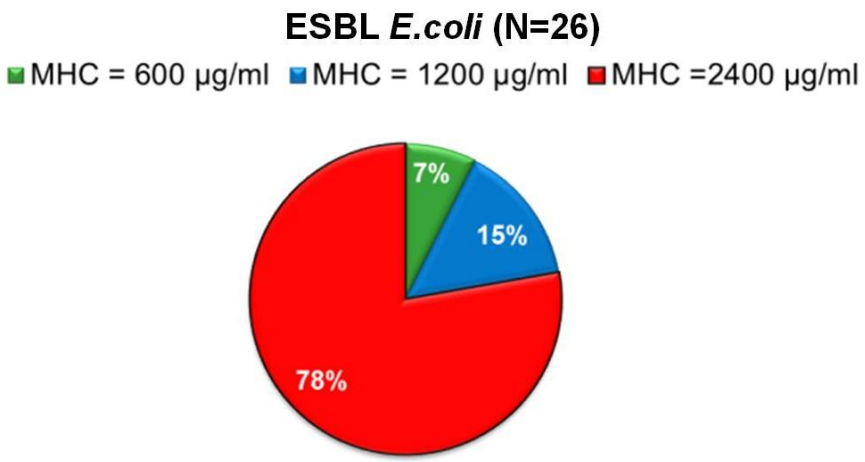
Activity of glyphosate on bacterial doubling time

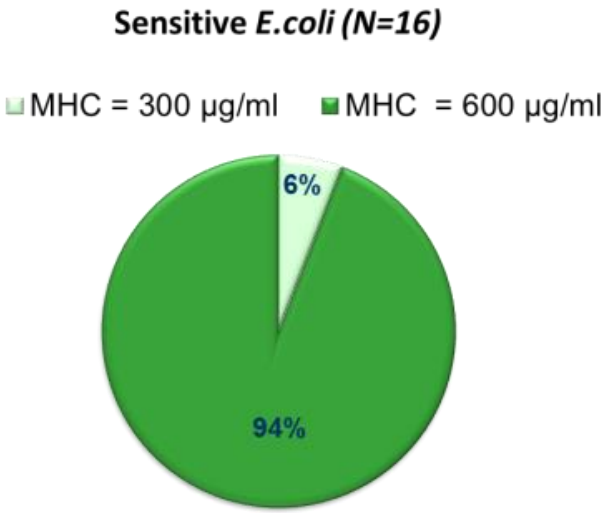


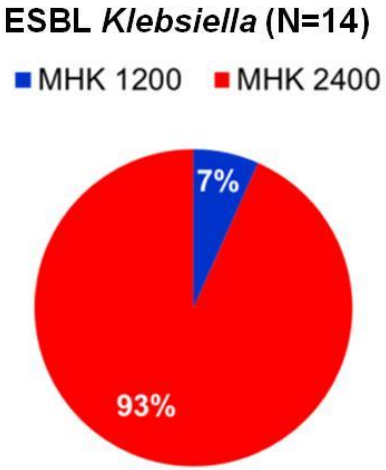
Fischer et al. 1986, J. Bacteriol. 186: 1147-1154

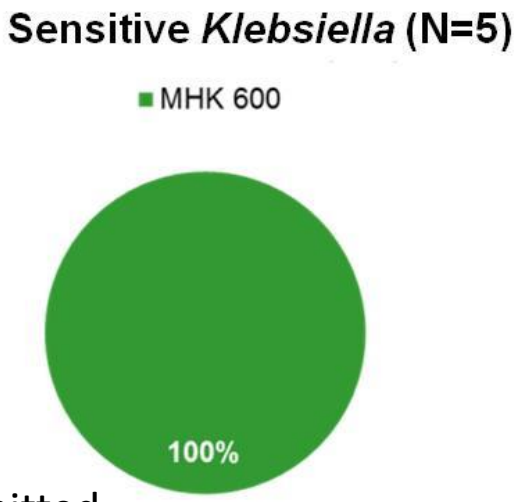
Lack of aromatic amino acids → feed back on shikimate pathway metabolites is staying away → toxic intermediates accumulate

ESBLs producing enterobacteria are glyphosate resistant too



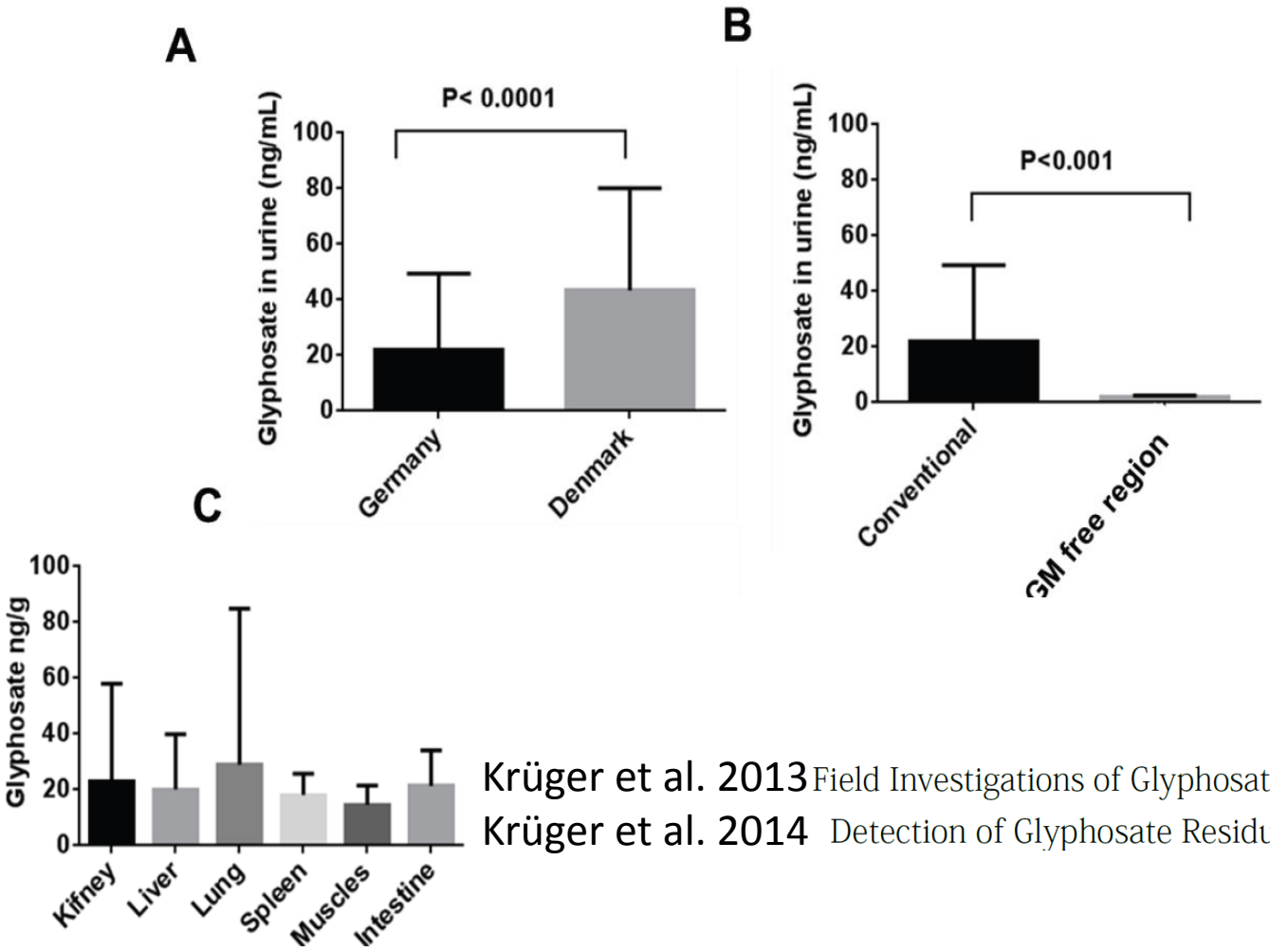






Krüger et al. 2014, submitted

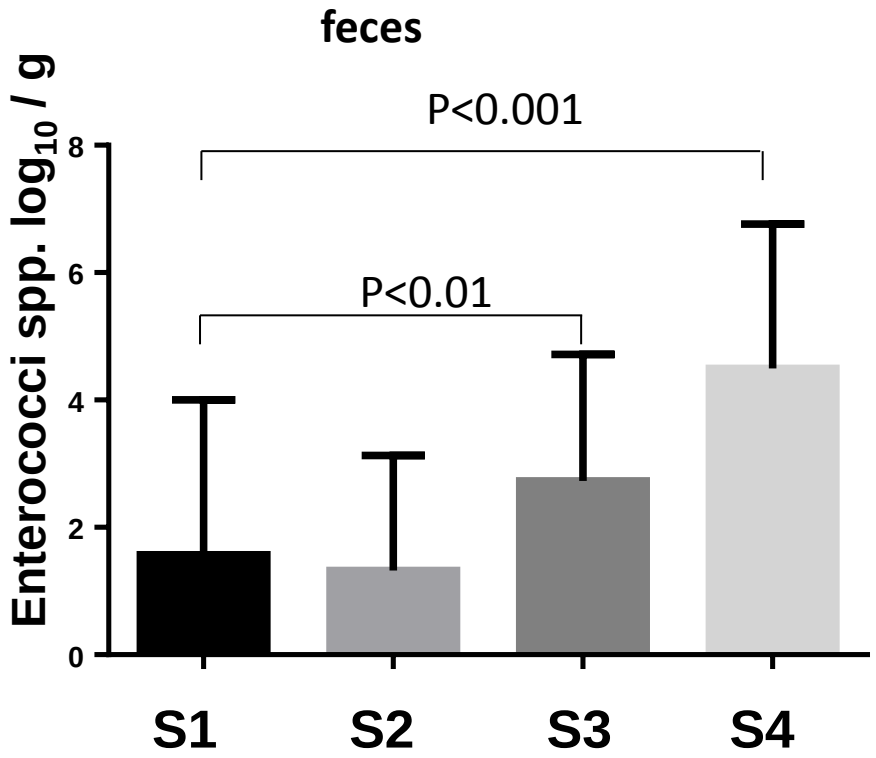
Is glyphosat detectable in excretions (urines) and organs/tissues of cows?



Krüger et al. 2013 Field Investigations of Glyphosate in Urine of Danish Dairy Cows

Krüger et al. 2014 Detection of Glyphosate Residues in Animals and Humans

Effects of glyphosate on bovine gastrointestinal microbiota (bacteria)



(Krüger et al. 2014, 10.1016/j.anaerobe.2014.06.010,)

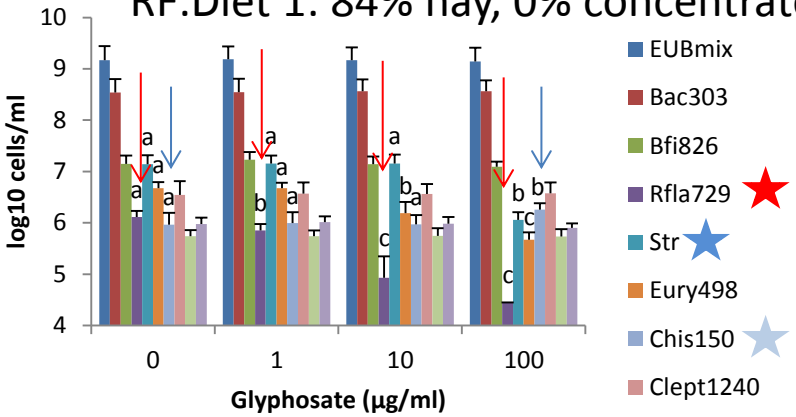
S1: start

S2: 4 weeks jerusalem artichoke sirup

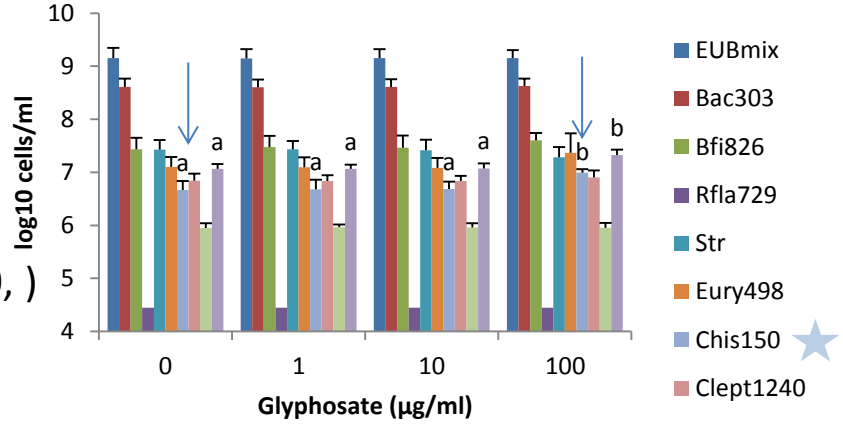
S3: 4 weeks probiotic enterococci

S4: 4weeks probiotic enterococci , stop of concentrated feed

RF:Diet 1: 84% hay, 0% concentrate



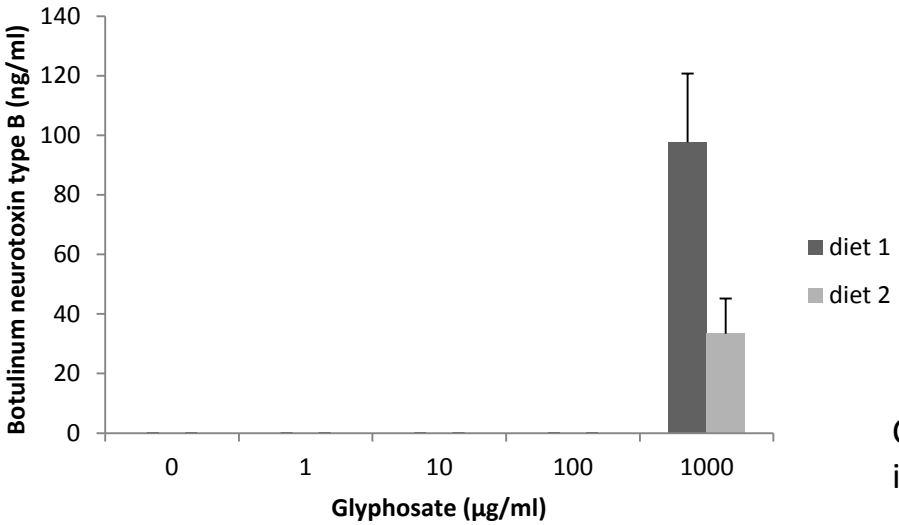
RF:2Diet 2: 60% hay, 40% concentrate



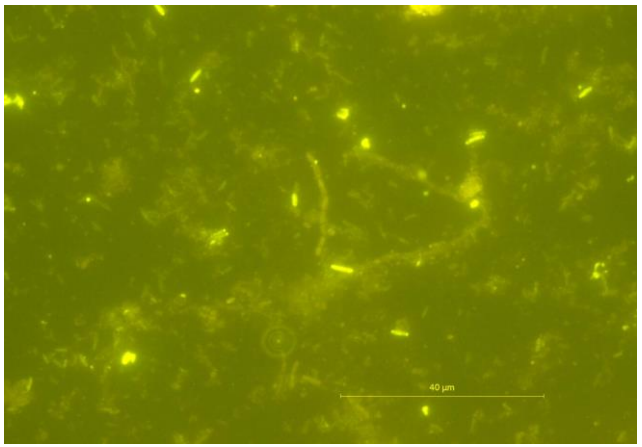
(Ackermann et al. 2014, submitted)

RF= rumen fluid

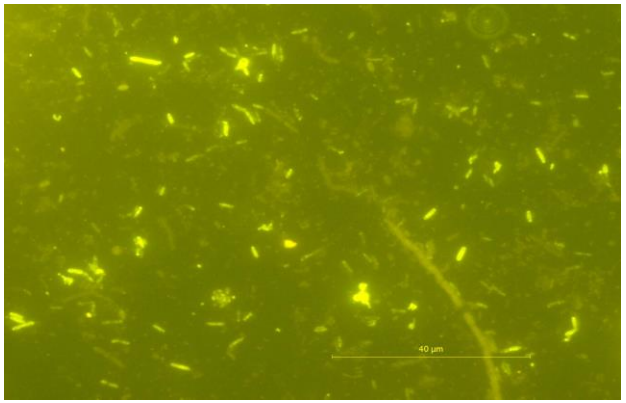
Effects of glyphosate on bovine gastrointestinal microbiota (clostridia)



Ackermann et al. 2014, submitted

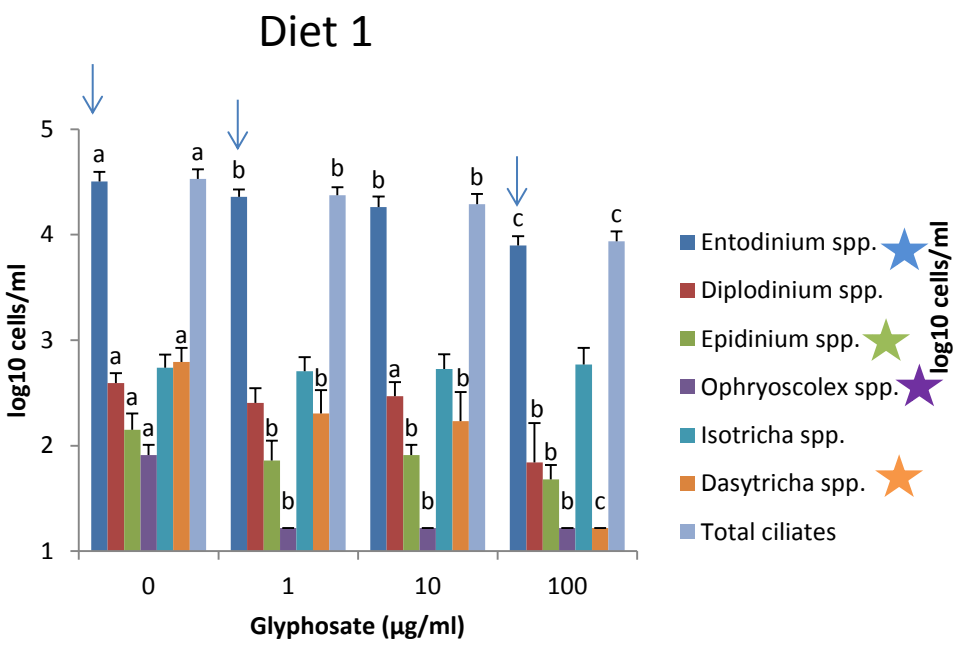


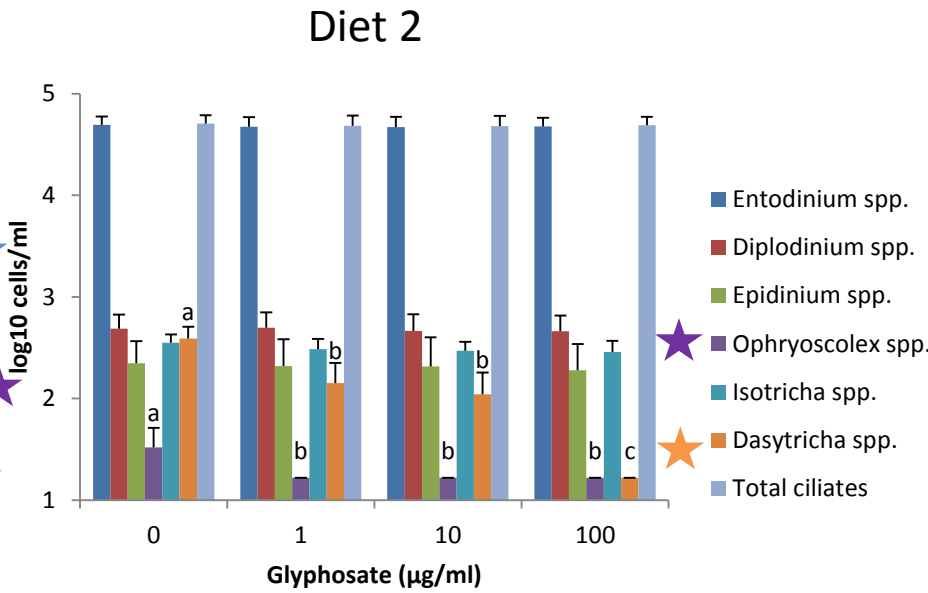
Chis150 with diet 1 at 0 µg/ml glyphosate after 48 h in vitro incubation



Chis150 with diet 1 at 10 µg/ml glyphosate after 48 h in vitro incubation

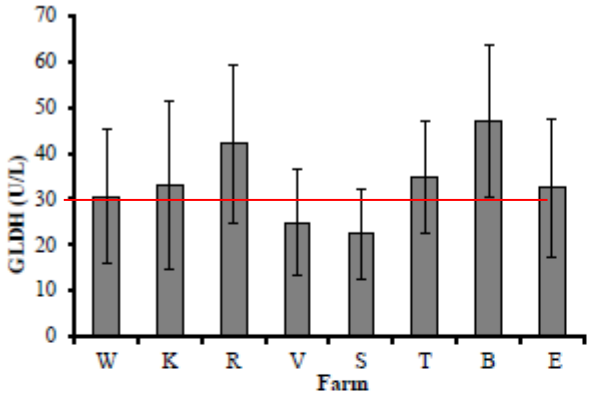
Effects of glyphosate on bovine gastrointestinal microbiota (protozoa)

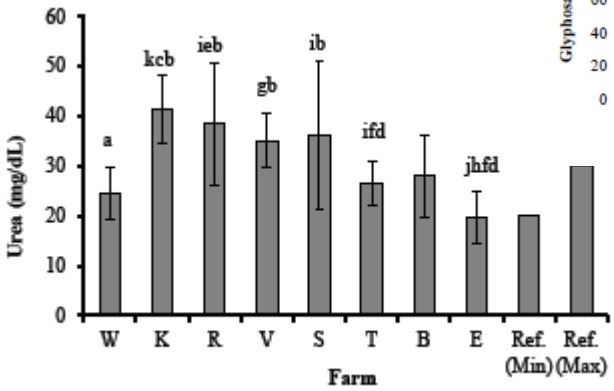


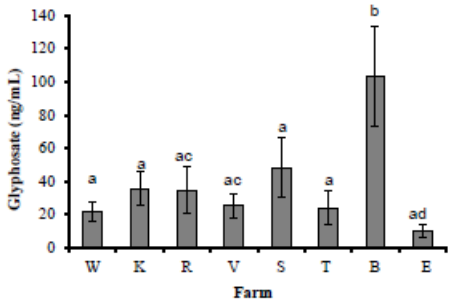


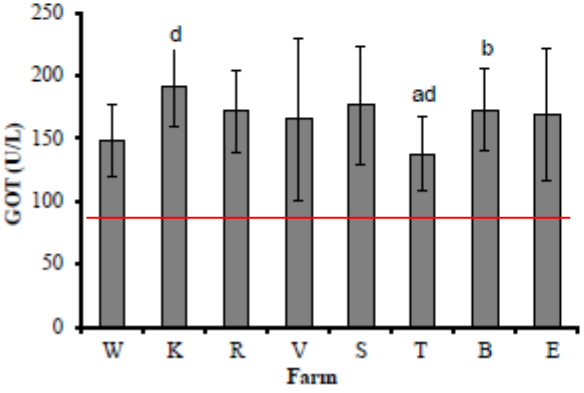
(Ackermann et al. 2014, submitted)

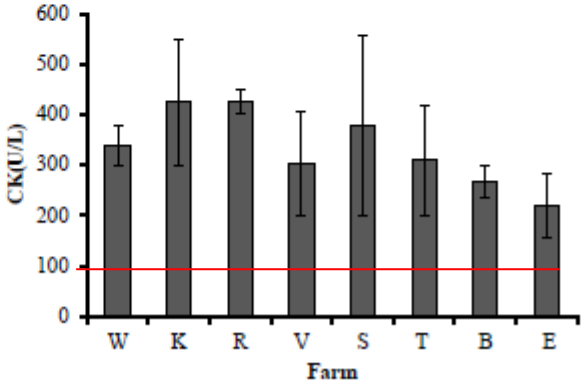
Effect of glyphosate on enzymes, and substrates







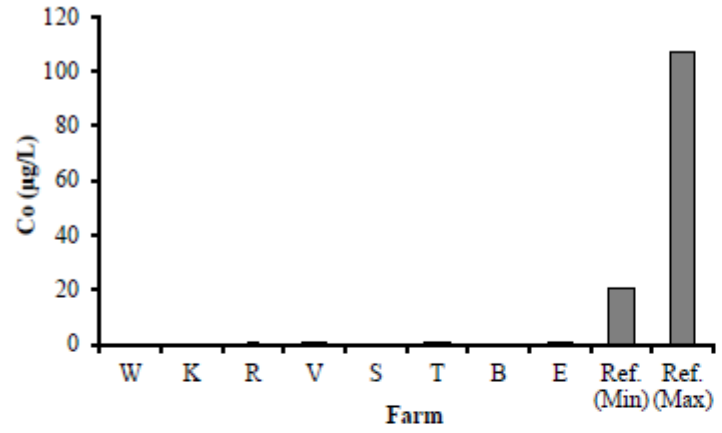


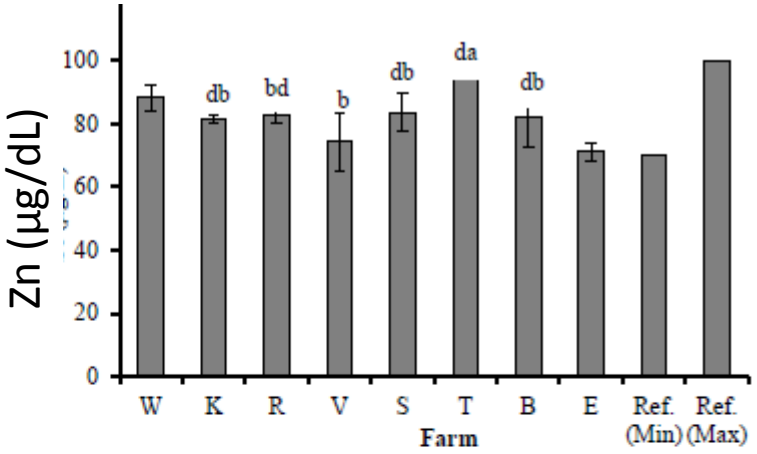


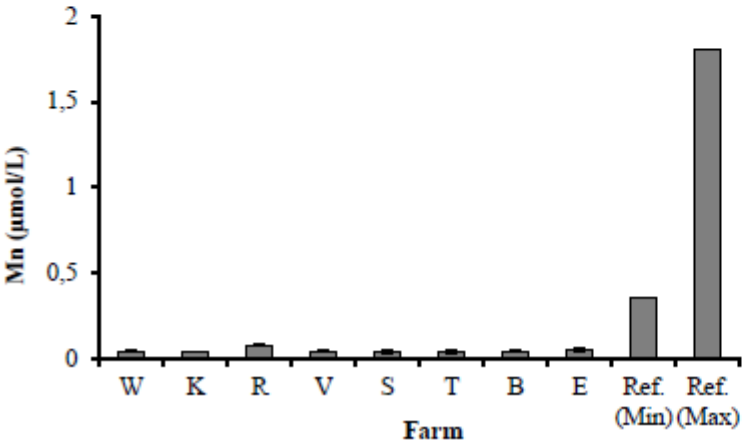
(Krüger et al. 2013 Field Investigations of Glyphosate in Urine of Danish Dairy Cows

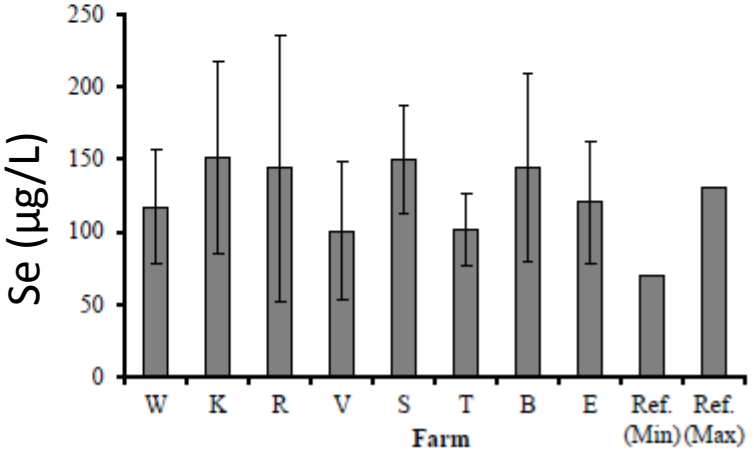
GLDH= *Glutamate dehydrogenese*, GOT=*Glutamate Oxalacetate Transaminase*

Effect of glyphosate on trace elements of Danish cows



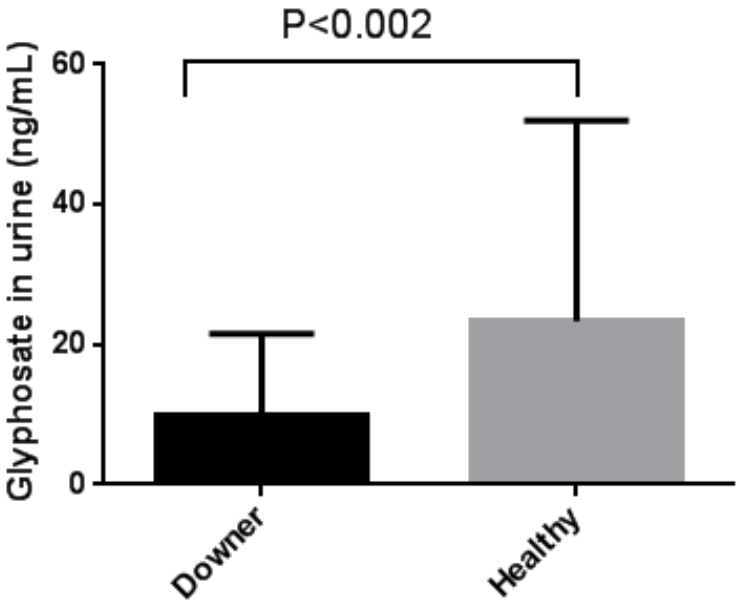




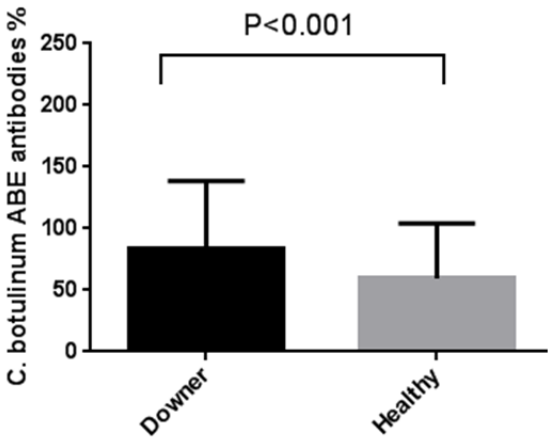


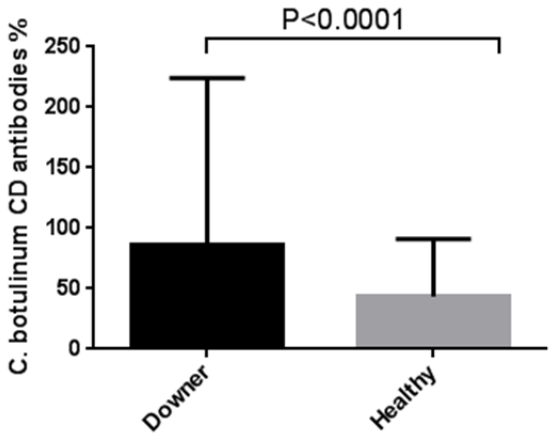
Krüger et al. 2013 Field Investigations of Glyphosate in Urine of Danish Dairy Cows

Downer cow syndrome and detection of glyphosate and *C. botulinum* antibodies



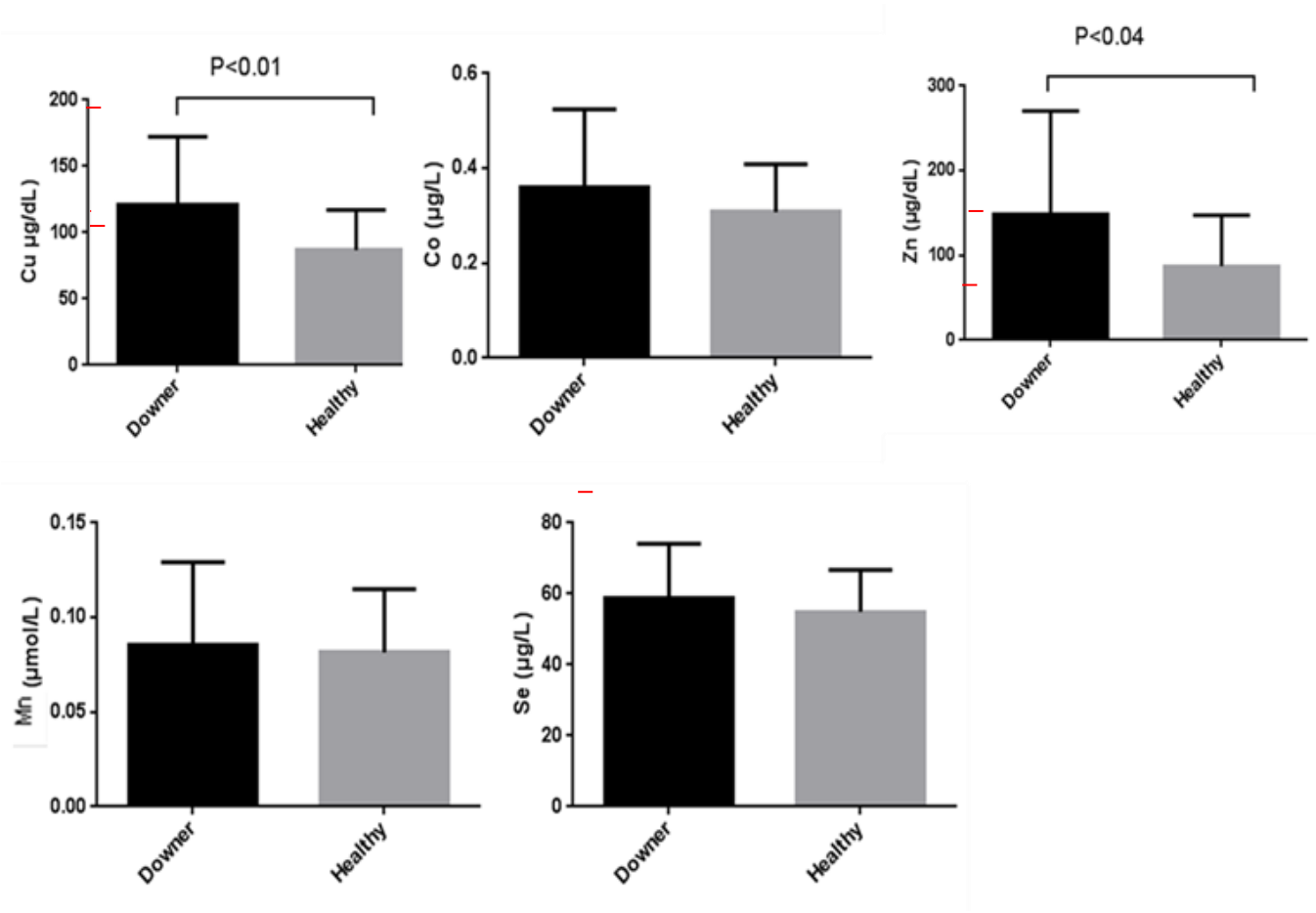
Glyphosate in urine of downer (N=52) and healthy (N=206) cows.





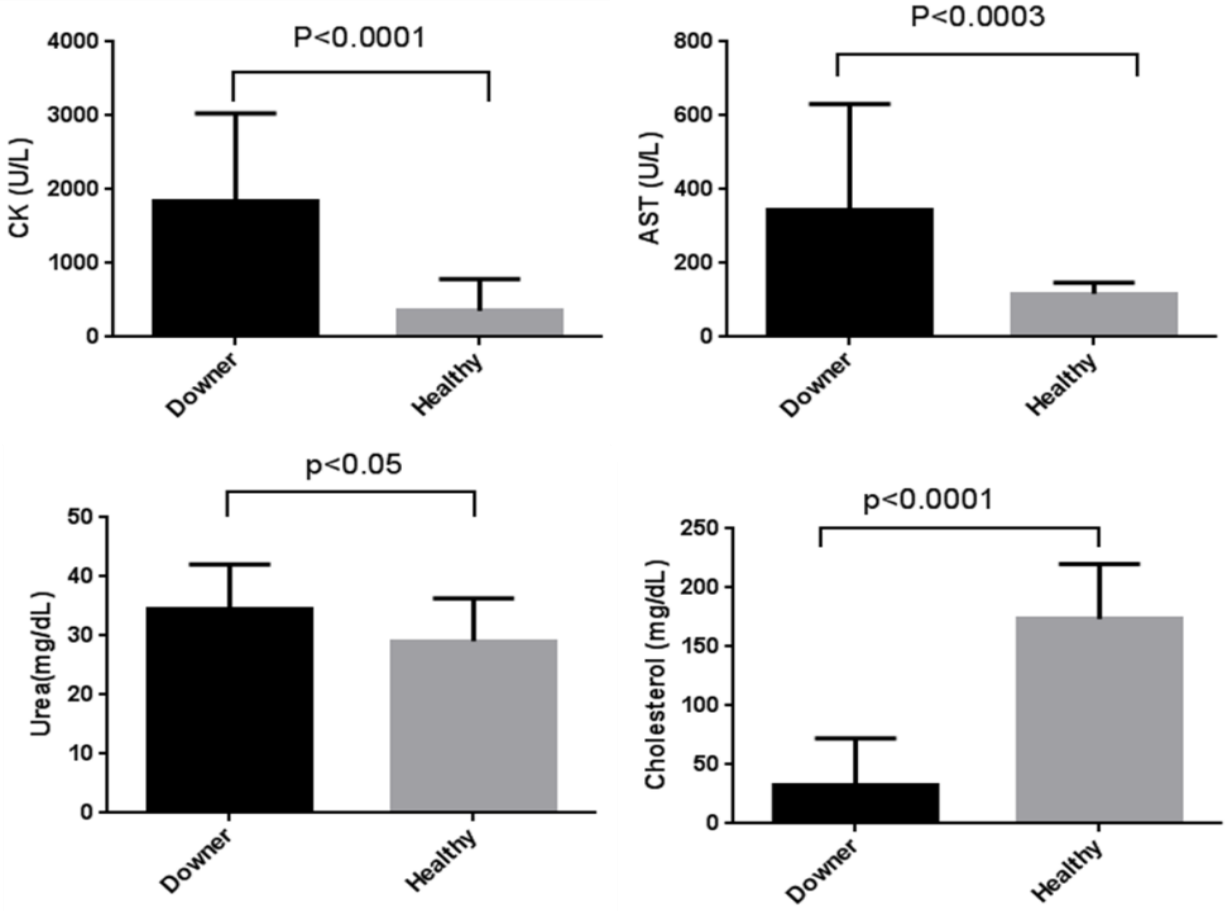
Rulff et al. 2014, in review

Trace elements in blood serum of downer cows



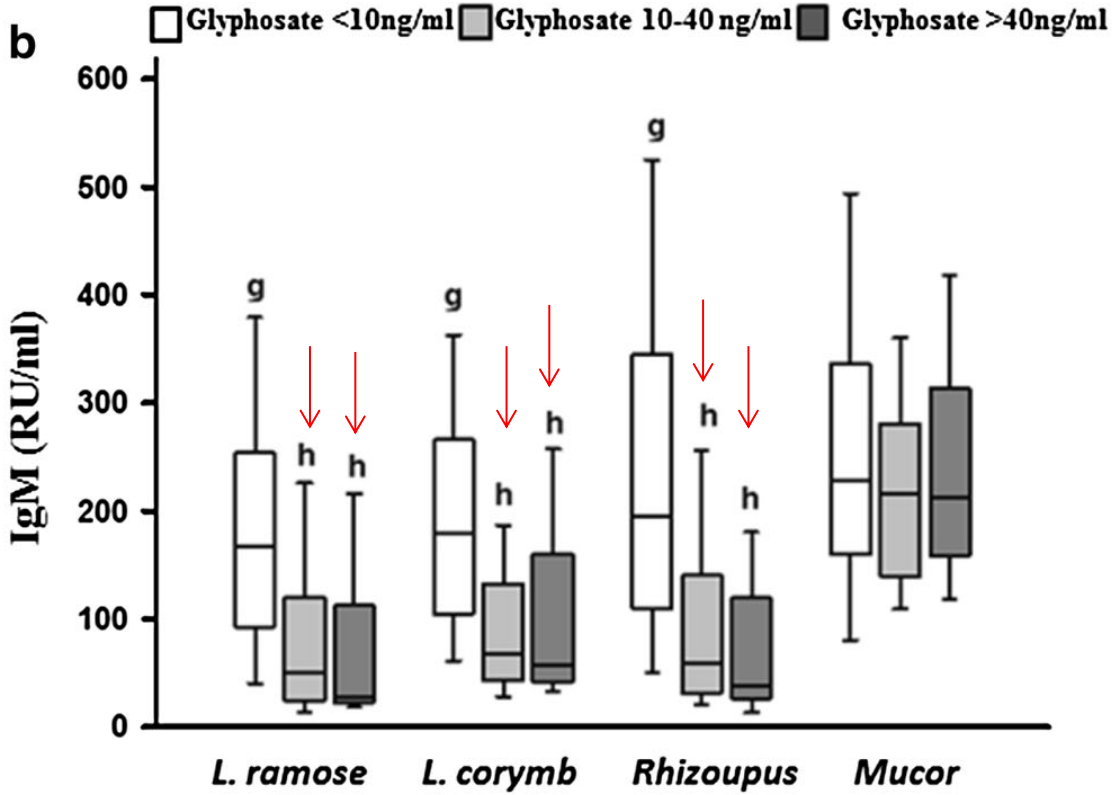
Rulff et al. 2014, submitted

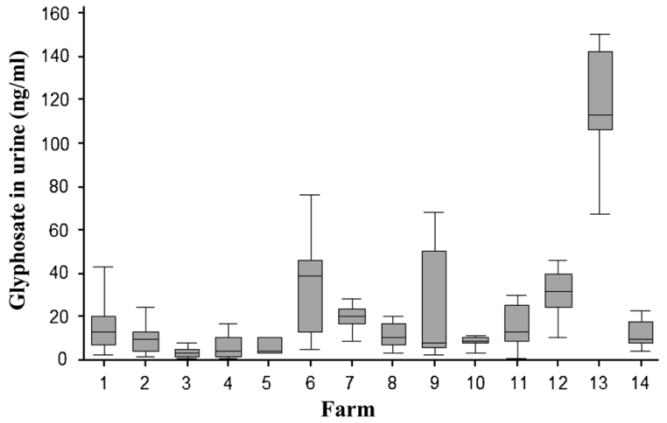
Substrates and enzymes in blood serum of downer cows



Rulff et al. 2014, submitted

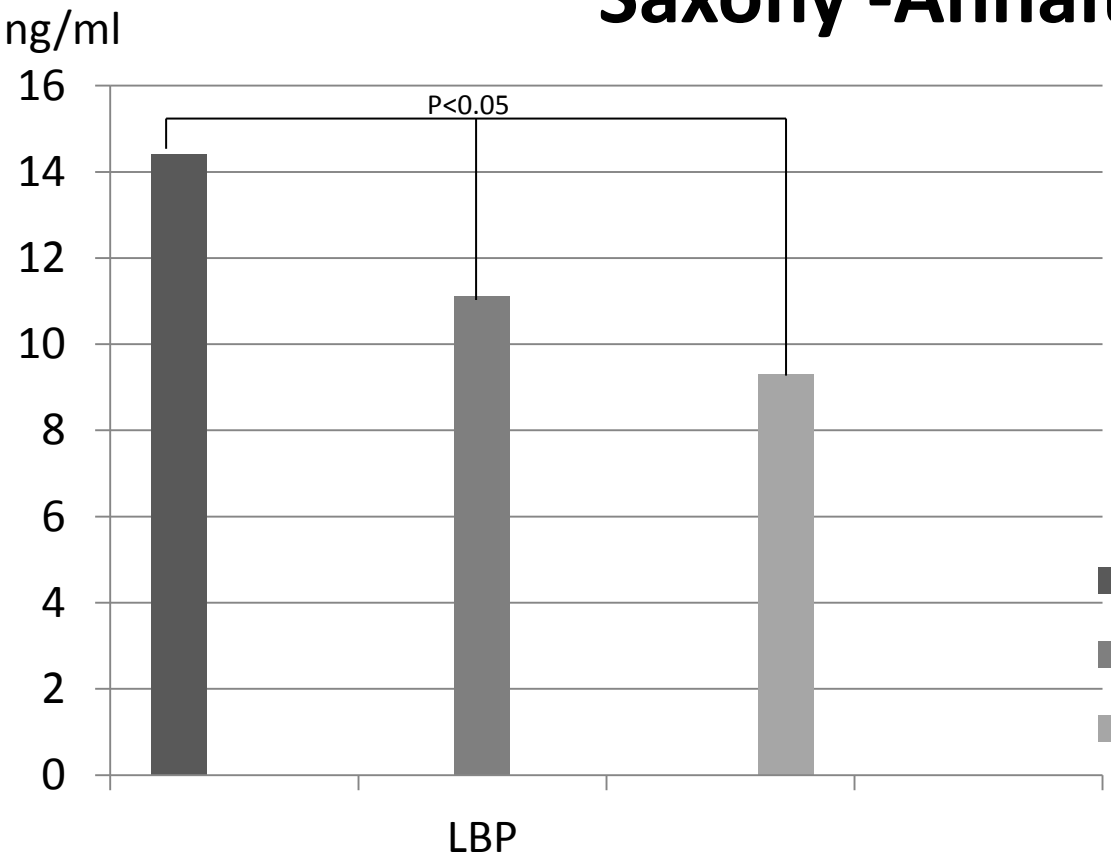
Glyphosate excretion by urine in relation to serum anti-*Mucorales* IgM antibodies of dairy cows in Saxony –Anhalt

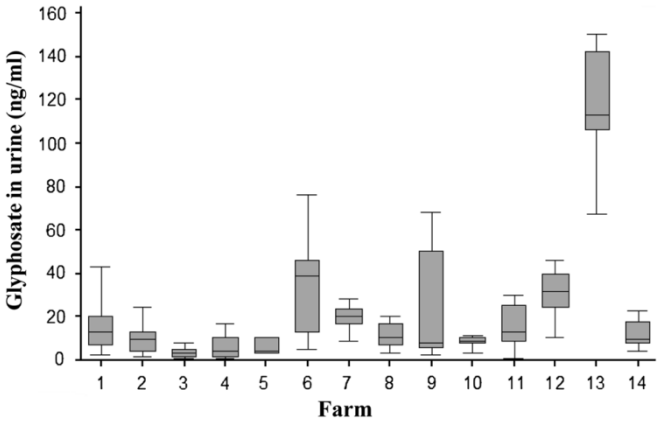


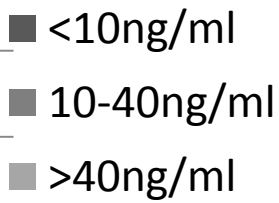


(Schrödl et al. 2014)

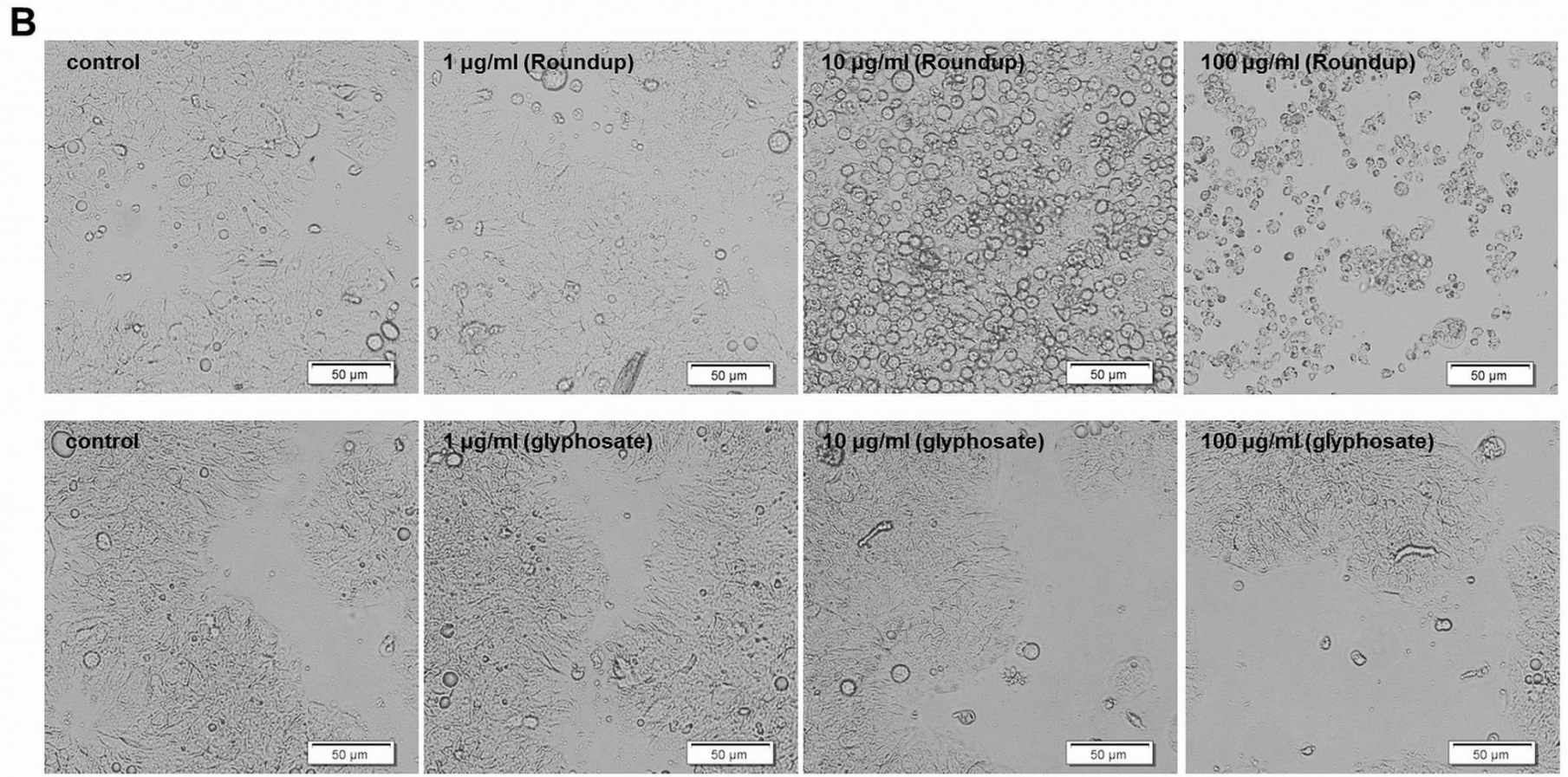
Glyphosate excretion by urine in relation to the acute phase protein LBP of dairy cows in Saxony -Anhalt





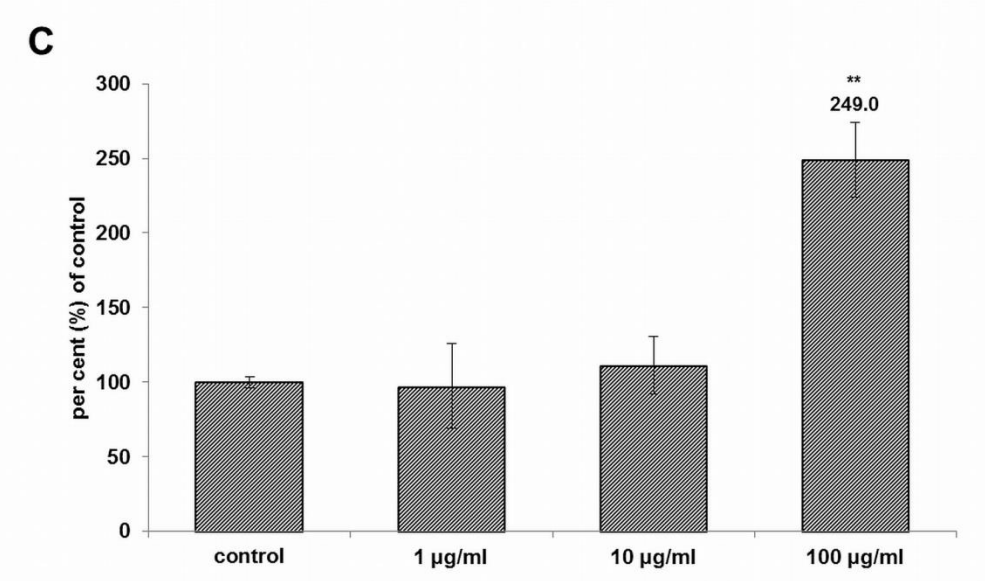


Toxicity of glyphosate and Roundup on HT29-MTX cells (48h)



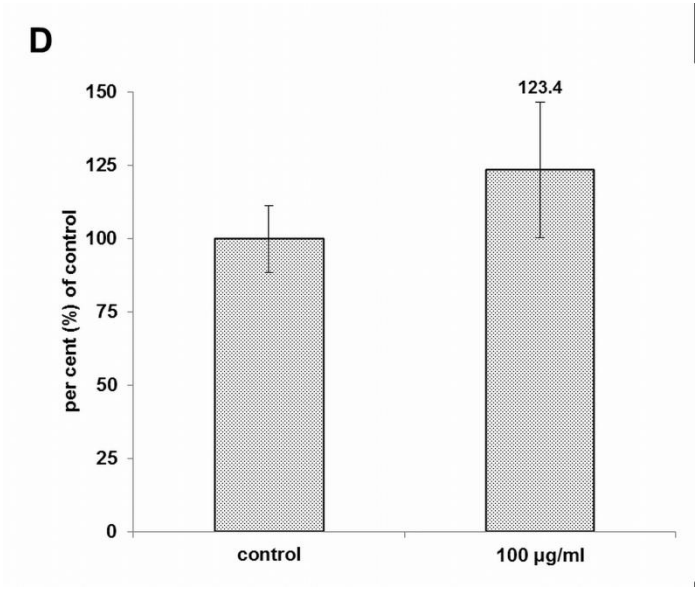
(Gac et al. 2014 submitted)

Induction of apoptosis by Roundup and glyphosate



The activation of apoptosis in monolayers (HT29-MTX) stimulated with high amounts of Roundup for 48 h. **p < 0.001, in comparison to the control group

(Gac et al. 2014 submitted)

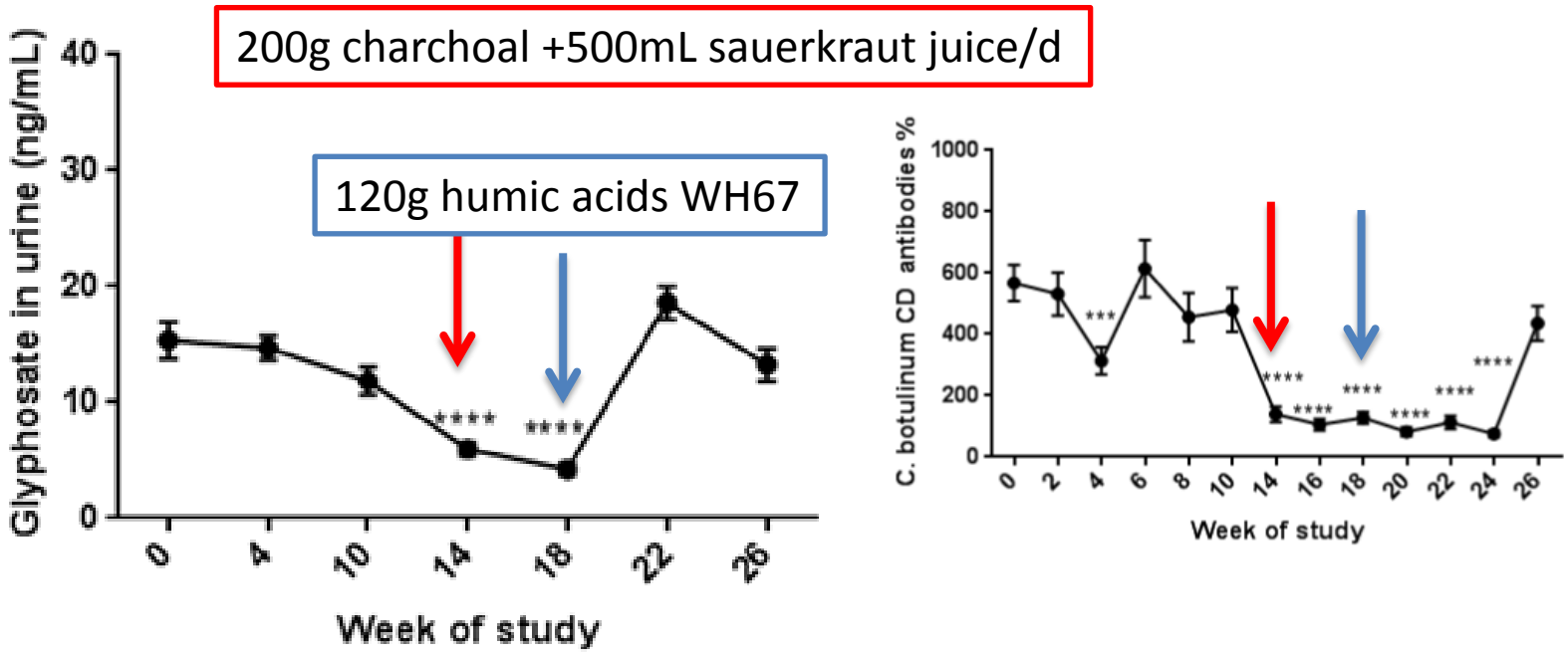


The effect of 48 h stimulation of cells with pure glyphosate solution at 100 µg/ml.

Neutralization of glyphosate

Shehata et al. 2013

Neutralization of the antimicrobial effect of glyphosate by humic acid *in vitro*



Gerlach et al.
J Clin Toxicol 2014

Oral Application of Charcoal and Humic acids to Dairy Cows Influences *Clostridium botulinum* Blood Serum Antibody Level and Glyphosate Excretion in Urine

Detection of glyphosate in organs and muscles of Danish malformed live born piglets

	Minimum	Maximum	Mean ±SD
Lung (N=38)	0.15	80	7.7 ± 18
Liver (N=38)	0	29.25	2.1 ± 2.2
Kidney (N=38)	0.1	38	3.2 ± 1.8
Muscles (N=38)	4.4	6.4	4.9 ± 1.8
Brain (N=38)	0.4	19.7	3.1 ± 4.1
Intestin (N=12)	0.7	7.7	2.4 ± 19
Heart (N=8)	0.4	80	12.9 ± 29.8

(Krüger et al. 2014)

Malformed alive born Danish piglets

Thank you for attention

- PD Dr. Schrödl
- Dr. Shehata
- Vet. S. Krüger
- Vet. Müller
- Vet. Kotsch
- Vet. Ackermann
- Vet. Neuhaus
- Vet. Schledorn
- Vet. Rulff
- Dr. M. Gac
- Prof. Rodloff

Alma , survivor of farm K, living 2 y in a home of elderly cows, died 26.08.14