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# Effects of mycotoxin binders on serum levels of vitamins and minerals in pigs

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# 1 Introduction

Having grown up in a rural area of Germany, in the so called pig belt, where there is more livestock than people, I have come into contact with mycotoxins early on. But I might have not understood the economic significance they pose. Is it possible for them to totally change the outcome of a fattening period, may it be because of the loss of animals or just their negative effect on growth, reproduction and feed intake.

Therefore, it is crucial to try and produce feedstuffs free from mycotoxins. But this poses a difficult task, as the recent years with their droughts have posed a burden on our farmers. Many had to rely on stored feedstuffs, which were a few years old, and could have accumulated mycotoxins through inadequate storage. Others had to harvest and use feedstuffs that they would have discarded or treated otherwise. Even if we now got a wetter summer, this wouldn't solve the problem of feedstuffs contaminated with mycotoxins, as wet weather at certain times of the year also promote the growth of mycotoxins on or within the crops.

For that reason, many farmers choose to be proactive and they add mycotoxin binders to their feedstuffs. This is a highly efficient way of binding mycotoxins in the intestines and eliminating them before they could be absorbed and cause harm within the body. This does not sound to be that much of an issue, but for a long time now, there have been rumours, that these binders do not only bind mycotoxins, but also reduce the availability of other minerals and Vitamins in the blood. And thereby reducing the beneficial effects of vitamins, this may alter reproductive parameters and therefore have an economic impact.

Therefore Biochem, a company that produces and distributes feed additives of all kinds set up a trial to test these rumours.

The hypothesis of this thesis is, that there is no significant difference between the means the three groups fed with basal diets combined mycotoxin binders and a control group fed with nothing but the basal diet.

# 2 Literature review

As discussed throughout many scientific studies ingestion of food stuffs and animal feed containing highly contaminated levels of mycotoxin can lead to a variety of undesirable or even deleterious effects for both animals and humans. In the scientific and agricultural world there have been several studies investigating the most efficient and practical ways to combat the effects of mycotoxins. These targets for application may fall under three broad umbrellas:- prevention of mycotoxin contamination of feed, detoxification/ removal of mycotoxins from infected feed and reducing the levels of mycotoxin absorption in the gastrointestinal tract.

Since the late 1980s/early 1990s research and development of new decontaminant strategies has been greatly popular as an aim at aiding agriculture in reducing the burden of mycotoxin in feed. Many processes include decontamination via either physical, chemical or biochemical means. According to Bata and Lásztity's review of decontamination in 1999 any decontamination process to reduce the toxic levels of mycotoxins must:-

- destroy, inactivate, or remove mycotoxins;
- not produce or leave toxic and/or carcinogenic/mutagenic residues in the final products or in food products obtained from animals fed decontaminated feed;
- not adversely affect desirable physical and nutritional properties of the product;
- be capable of destroying fungal spores and mycelium
- be technically and economically feasible.

## 2.1 Chemical Adsorbents

As previously discussed, the economic and public health impacts of higher than acceptable levels of mycotoxin contamination not only effects productivity but also poses a risk to the consumer. As a result, to improve food safety effective and practical approaches to detoxifying feed must be investigated. The European Food Safety Authority have strict guidelines and testing to be followed before a substance can be licensed as a food additive for reducing mycotoxin contamination. Guidelines stated that thorough in vitro and in vivo tests should be carried out to evaluate binding efficacy.

During early research many investigations were carried out in vitro concluding that the majority of mycotoxins could be bound by at least one adsorbent. However, as the research initially focused on singular mycotoxin studies it became apparent that focus should be shifted to studies dealing with several mycotoxins of varying structure as these often appeared together in contaminated feedstuff. Several studies have also suggested that co-existing mycotoxins have additive effects and potentially toxic interactions within animal species and human (Clarke et al., 2014). Thus, the removal of several co-existing mycotoxins from the feed is essential. Studies have shown the most practical approach to counteracting mycotoxins is to alter the bioavailability of the feed toxins in the gastrointestinal tract thus inhibiting the absorption of the mycotoxins. The most common approach involves the addition of nutritional inert adsorbents to the livestock diet. Initial studies investigated previously unanticipated benefits of anticaking HSCAS clays finding that such feed additives could prevent hepatotoxic effects of mycotoxins (Phillips et al., 1988). This led to an increase in research towards use of other clays against mycotoxin damage. Activated carbon (AC), hydrated sodium calcium aluminosilicate (HSCAS), a variety of clays and other miscellaneous adsorbents have been analysed and deemed most practical additions to livestock diets (Huwig et al., 2001; Kabak et al., 2006) The sorption capacity of many of these clays and other adsorbents are directly related to their physical structure, thermodynamic capabilities and modified forms.

### 2.1.1 Activated charcoal

The principle of using activated charcoal/carbon as a method for combating poisonings has been used for centuries. This more rudimentary adsorbent is formed from the thermal decomposition of several organic compounds. Importantly this irreversible pyrolysis creates a non-soluble powder which is capable, upon undergoing a further activation process, of

becoming a highly porous structure which remains in the gastrointestinal tract. The stability of activated charcoal within the liquid environment of the gastrointestinal tract is one of the key features enabling adsorption and removal of toxins. The ability of the charcoal to remain within the gastrointestinal tract without dissociation and to bind and remove mycotoxins has been a practical approach to reducing mycotoxin burden. A wide range of activated charcoals can be obtained via processing and activation of many organic compounds, many of which are often created as a secondary product during industrial processing. This range of activated carbons can absorb a wide variety of organic compounds with varying pore size.

In vitro studies have shown that other key physicochemical parameters can affect the binding ability of activated charcoal (Ramos et al., 1996; Galvano et al., 1998; Wielogórska et al., 2016). The absorption capability of charcoals can depend upon pore size of the charcoal, surface area, chemical nature of the mycotoxin, charcoal dose, pH and the presence of gastrointestinal content/feed mix. Many studies discuss the use of experimental activated carbons (EACs) and commercially activated carbons (CACs). Depending upon the original material to be thermally decomposed, e.g. Oil residues, lignin or food shells or stones, the surface area measurements for CACs may vary from 500 $m^2/g$ to over 2,000 $m^2/g$. Superactive Charcoals have also now been developed which have greatly increased surface area (up to 3,500 $m^2/g$) which exponential increase the efficacy for mycotoxin absorption (Ramos et al, 1996). In vitro study by Galvano et al assessing the efficacy of commercially activated carbons concluded that the most efficient ACs expressed high affinity for binding to several of the most widespread mycotoxins. Their results indicated that the same five ACs (CAC1, CAC2, CAC4, AF32, and AF48) could be seen to express high absorption results for $AFB_1$, $FB_1$, Ochratoxin A and DON (Galvano et al, 1998). Specifically, with regard to absorption of Ochratoxin A this study found that these top five ACs expressed a higher saturation level (80-121 µg OA/mg of AC) and therefore 30-45-fold increase in adsorption affinity than previous studies had concluded. Interestingly, the same in vitro study found that at low levels of DON mycotoxin contamination (test 4 with 2mg of sorbent) very poor adsorption levels were observed across all ACs. This perhaps suggest that at contamination levels lower than those registered as harmful the benefits of addition of ACs to feed is negligible. It could perhaps then be postulated that ACs will only be of benefit in addition to feed which suffers from a high non-specific mycotoxin burden. Conversely some studies have questioned the effectivity and therefore use of activated carbon in vivo. For example, Rotter et al. (1989) suggested that charcoal supplementation of up to 10,000 ppm in

combination with an Ochratoxin A contaminated diet had no beneficial effect when given to Leghorn chicks (Rotter et al., 1989). They suggest that perhaps such beneficial effects of binding as seen in vitro are not correlated with in vivo studies. Whether this theory is still the case after 30 years remains to be investigated.

Another study specifically looking at the intestinal absorption of the trichothecene group of mycotoxins in pigs compared the absorption with and without the presence of activated carbon as one of 14 absorbent materials assessed (Avantaggiato, 2004). This study used a modelling system to simulate the intestinal tract of healthy pigs finding that the majority of deoxynivalenol (DON) and nivalenol (NIV) uptake from contamination feed source occurred in the jejunal compartment of the gastrointestinal system. When testing the binding ability of all 14 absorbent materials in the same pH range it was found that only activated carbon bound enough mycotoxin per gram to be deemed as high affinity for the trichothecene mycotoxins (35.1 µmol and 8.8 µmol DON and NIV/g adsorbent, respectively). The addition of 2% activated carbon to a contaminated wheat source significantly reduced the level of mycotoxin absorption. Absorption reduced from 51% to 28% for DON and from 21% to 12% for NIV.

However, it has been suggested that perhaps this high affinity to such a broad spectrum of mycotoxins although highly efficient may not be practical or even safe in terms of addition into nutritional feed.

## 2.2 Mineral adsorbents

### 2.2.1 Aluminosilicate clays

Silicon and aluminium are two of the most prevalent minerals, they combine with oxygen to form tetrahedral structures which polymerise. Such a wide variety of mineral structure can occur due to the complexes formed by polymerisation. The main driving force behind this diversification is the oxygen-rich tetrahedral geometry, a silicate tetrahedron will favour formation of a less dense crystal structure which tends to polymerise. Stages of further clay weathering reveal a transformation into a mineral group; this clay-size mineral group includes Vermiculite, Montmorillonite, Kaolinite. The Aluminosilicates can be further divided into two subclasses, according to their spatial structure: phyllosilicates and tectosilicates.

Phyllosilicates can bind cations within their structure creating a variety of classes of mineralogy. When octahedral sheets are populated by divalent cations (e.g., Mg2+ or Fe2+) the clays are termed trioctahedral or dioctahedral when sites are occupied by trivalent cations (e.g., $Al^{3+}$ or $Fe^{3+}$) (Bleam, 2017). The lamellar layer structure of mineral groups is filled with water molecules and interchangeable cations. Hydration of interlayer cations forces clay layers apart this swelling ability has been suggested as a method of binding for mycotoxins.

Hydrated sodium calcium aluminosilicate (HSCAS)

HSCAS could be said to be the most extensively researched adsorbent due to the high number of scientific studies assessing the absorbent's high affinity to aflatoxin. Many scientific studies have assessed the use of a processed calcium montmorillonite originally added to feed as an anticaking agent. Specific studies considered the effects of HSCAS aflatoxin binding in poultry. An initial study by Phillips et al proved that HSCAS binds aflatoxin B1 with high affinity and high capacity to protect broiler chicks from negative effects of scientifically formulated aflatoxin contaminated diets (Phillip et al., 1988). This study compared results from parallel in vitro and in vivo studies. The in vitro trial measured the sorption of a variety of adsorbents using measured decrease in radioactivity of a supernatant after the addition of the sorbent to a radioactive aflatoxin. The trialled adsorbents were aluminas, zeolites, silicas, phyllosilicates and chemically modified phyllosilicates. The results of this study showed that all 38 adsorbents were capable of binding aflatoxin however

with varying success and capacity. HSCAS and pyrophyllite were proven to show the highest absorption capacity of all those tested at over 80% aflatoxin binding. The strength of this binding was then further tested by simulated extraction using solvents. Less than 10% of bound aflatoxin was extracted from the HSCAS-toxin complex, this suggested a strong bond formation. The in vivo study analysed the addition of 0.5% HSCAS to aflatoxin contaminated soybean meal sorghum diet. The paper has shown dietary HSCAS to decrease the growth inhibitory effects of aflatoxin which were seen in the liver at necropsy of those individuals feed 7.5mg/kg aflatoxin contaminated feed. In both the broiler and Leghorn chicks protective effects were observed as livers sampled appeared normal when fed a diet including HSCAS (Phillip et al., 1988). Building on these initial studies further papers in 2002 looked specifically at the chemisorption of aflatoxins and the structural modelling of the binder-mycotoxin complex. It has been suggested that the interlamellar region of HSCAS is the strongest site for binding due to an electron donor acceptor mechanism, the exterior surfaces of the clay were responsible for only minor sorption of aflatoxins (Phillips et al., 2002).

Further studies assessed the binding ability of HSCAS to other mycotoxins. Diets containing ergot contaminated fescue where applied to in vitro studies. It was shown that HSCAS removed more than 90% of ergotamine from aqueous solution (Chestnut et al., 1992). However, in vivo experiments within the same study found that HSCAS did not protect sheep from mycotoxins responsible for fescue toxicosis and even went as far as to say that HSCAS negatively affected the absorption of Mg, Mn and Zn. Further evidence suggesting contraindications for use of HSCAS in feed was found in poultry nutrition studies. One paper found that the addition of HSCAS at concentrations of 0.5 and 1.0% significantly reduced zinc utilization (Chung et al., 1990). This study found a linear correlation between the percentage of HSCAS in test feed and tibial Zn levels. Total tibia Zn decreased by 5% and 14% respectively when the diet contained 0.5% and 1% HSCAS.

### 2.2.2 Smectite phyllosilicate clays

Smectite clays are classed as such if at least two-thirds of the octahedral sites are occupied by dioctahedral minerals. Montmorillonite is the most common smectite clay. Many of the smectite clays have a cation exchange capacity of between 60 and 150 cmolc $kg^{-1}$, layer charges and interlayers all of which are involved in bonding of mycotoxins (Bleam, 2017).

### 2.2.3 NSP (calcium-rich montmorillonite)

A hydrated Ca-montmorillonite (NovaSil Plus or NSP) is another proposed absorbent which has been extensively studied. The adsorption of toxic metals (As, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Zn), have been studied predominantly. Montmorillonite and its modified forms have much higher metal adsorption capacity compared to that of kaolinite as well as modified-kaolinite. In vitro studies of NSP have assessed the specific mechanism of binding for aflatoxin. NSP and NSP-carnitine were heated to 800ºC to initiate collapse of the lamellar structures. Aflatoxin B1 binding capacities of both collapsed structures were drastically reduced suggesting that the majority of mycotoxin binding occurs within the interlayer structure of these clays (Wang et al., 2017). This is consistent with findings from computational models of the modified clays. Species specific studies have often suggested NSP as being an effective binder for use in animal feed. A study by Maki et al 2016 investigated the effect of addition of NSP to dairy cattle diets therefore analysing the binder's effect on milk excreted aflatoxin. The aflatoxin excretion and transfer to milk were calculated based on daily milk production. This study indicated that cows consuming the binder inclusive diets at NSP-0.5% and NSP-1% excreted 11.86 μg/d and 7.38 μg/d aflatoxin in milk respectively. This resulted in a reduction of 51.3% and 69.7% aflatoxin levels in milk (Maki et al., 2016). As a result this study concluded feeding NSP as either 0.58% or 1.17% of the dry matter intake of the feed ration would prove an effective strategy for prevention of excretion of aflatoxin levels in dairy milk without any adverse effects on milk composition. Milk produced from the experimental animals was analysed for vitamin A, riboflavin and mineral composition. Data from this study showed consistent levels of minerals across all treatment groups. Vitamin A and Riboflavin levels within the milk produced remained consistently stable across all treatment combinations at around 0.28 and 1.57 μg/mL. This study perhaps could be used to support the theory that NSP as a mycotoxin binder does not bind vitamins or minerals as an adverse effect.

### 2.2.4 Bentonite

Bentonite is another common term for naturally occurring volcanic aluminium phyllosilicate clays. Bentonite is a general term for clays containing montmorillonite as their major component. In certain countries a clay composite is defined as bentonite if it contains more than 70% smectite. Montmorillonites as a group have a variety of differing layered silicate structures. The physical properties of these clays have been shown to vary depending upon binding sites and capacity at either it's external surfaces or within its interlaminar spaces,

Montmorillonites have been shown to have a high efficacy for binding to aflatoxin, one study binding 93.4 to 96.7% of aflatoxin from a buffer solution (Masimango et al., 1978). Species specific studies have shown bentonites to be efficient at binding aflatoxins across a range of species. Diaz et al. (2004) looked at the use of bentonite as a feed additive to reduce aflatoxin excretion in milk from dairy cows. Experiments in this case tested a range of varying structural bentonites. Results found that three sodium bentonites and one calcium bentonite significantly reduced aflatoxin contamination levels in milk by 61%, 65%, 50% and 31% respectively. An earlier porcine study assessed a variety of feed additive clays (0.5% clay) incorporated into an aflatoxin contaminated diet. The modified calcium bentonite assessed in this study was shown to improve performance parameters and serum biochemistry even restoring levels to that seen in normal non contaminated feed trials. Incorporation of 0.25-2% Ca-bentonite into porcine diet was shown to linearly increase average daily gain and average daily feed intake (Schell et al., 1993). A more recent review on bentonite by the European Food Safety Authority stated that "A range of 1-2 % bentonite in complete feed for laying hens and poultry for fattening seems safe" and that "ruminants seem to tolerate high levels of bentonite" however very little information appears to be available regarding safe dosing levels in other species (EFSA, 2013). This report therefore concluded that a limit of 2% of diet should be authorised for use of bentonite. When discussing interaction with other constituents in the diet the report concluded that in vitro data suggests the bentonite/sepiolite feed mix in question does not bind vitamins A and E and pantothenic acid and trace elements (Cu, Zn and Mn). However, there is a serious lack of in vivo study data to support this theory.

#### 2.2.4.1 Bentonite drug interactions

There are studies looking at the contraindications or side effects of use of bentonites as a mycotoxin binder. Part of one such study focused on a variety of mycotoxin binders and their potential drug interactions (Devreese et al., 2013). In this in vitro study passage of the macrolide antibiotic tylosin through porcine epithelial cells both with and without the presence of mycotoxin binders were evaluated. Presence of a bentonite clay in the jejunum of piglets was shown to significantly reduce the passage of tylosin across the epithelium compared to gluco-mannan. Levels of tylosin across the epithelial barrier were shown to reduce by 92% with the addition of the bentonite mycotoxin binder. Interestingly, data from this study suggested tylosin residues at the 12hr time point remained at well below 500ng/ml for all tested treatments. However, in the case of administration of tylosin alone and tylosin

with glucomannan increasing tylosin levels were seen at 24, 36 and 48hrs post application. Levels of tylosin remained stable at well below 500ng/ml for all time points in the case of bentonite administration, suggesting a binding ability. This supports the theory that bentonite should not be used in conjunction with tylosin medicated feed or water as the antibiotic efficacy is almost totally removed. This drug interaction is concerning as it can lead to complete antibiotic therapy failure and perhaps promote antibiotic resistance to tylosin within pigs. Further studies have also discussed the possible side effects of mycotoxin binders prior to antibiotic administration rather than just concurrently with antibiotics, growth promoters or coccidiostats. One such in vivo study involving broiler chickens found that addition of a bentonite clay alongside tilmicosin rendered the antibiotic protection ineffective. Addition of 2% bentonite in the feed ration led to reduced effectivity of the lowest administered tilmicosin dose (300 g/ton) and increased incidence of air sac lesions (Shryock et al., 1994). Other broiler studies looking at the interaction with lincomycin antibiotic found a significant reduction in maximal plasma concentration for the antibiotic when pretreated with a binder inclusive feed rather than control feed, $3.27 \pm 0.15$ $\mu g \cdot mL^{-1}$ and $10.65 \pm 0.17$ $\mu g \cdot mL^{-1}$ respectively (Amer, 2015). As a result, many of such studies have led to the prevention of use of bentonite alongside administration of veterinary medicinal products.

Not only have drug interactions been reported with bentonite but suggestions of conflicting undesirable vitamin and mineral binding have been published. One such study extracted calcium montmorillonite from bentonite to explore the effectivity of the binder against mycotoxicity in broilers. This study suggests that binders can affect levels of mineral deposits in bone when used in animal feed. The data from this study showed significant differences in mineral content of broiler bones after use of a montmorillonite binder. Specifically deposits of Manganese where shown to fall from 35.6mg/kg to 23.8mg/kg, Fluoride decreased from 311.2mg/kg to 217.8mg/kg and Lead from 120.5 to 108.3mg/kg after the addition of the mycotoxin binder to the diet (Desheng et al., 2005). When put into context this would suggest that in the case of broilers Mn would need to be supplemented in the diet if montmorillonite were to be used as a feed additive.

### 2.2.5 <u>Zeolite</u>

Zeolite is another crystalline hydrated aluminosilicate with alkaline cations binding to form it's lamellar structure. Zeolites can either be natural or synthetic aluminosilicates. The majority of studies have looked at the beneficial effects of zeolite in broiler chicken feed. Scheideler assessed the effects of feeding zeolite to broiler chicks alongside 2.5mg/kg aflatoxin contaminated feed. It was found that addition of zeolite helped to minimise the negative effect of aflatoxin on serum calcium and inorganic phosphorus levels (Scheideler, 1993). Further studies were undertaken to not only assess a synthetic zeolite efficacy in aflatoxin binding but also serum biochemical and haematology components (Kececi, 1998). Results of this study supported other studies showing that 5mg/kg zeolite caused uric acid levels to remain similar to that in a control diet.

However conflicting studies have shown no beneficial effects of addition of zeolite in broiler feed. Infact, some go as far as to say there may be contraindications in use such as interference in mineral distribution. One such study looked at the effects of addition of Ethacal Feed Component, a trade named sodium aluminosilicate, to chick and layer hen feed. This study indicated addition of zeolite to a 1% nonphytate P diet leads to a significant reduction in egg production, egg weight, and feed efficiency. They suggest that the binder may affect P utilisation by poultry (Moshtaghian et al., 1991).

### 2.2.6 <u>Sepiolite</u>

Sepiolite is a porous magnesium silicate clay often used as a pelleted feed additive. In vitro efficacy studies with regard to sepiolite have shown that at 2% of the feed ration the binder was able to adsorb 86.7% of aflatoxin B1 (Masimango et al., 1978). This shows how effective the binder is at removal of aflatoxin in vitro, an effect which is assumed to be mimicked in vivo. One such in vivo experiment compared performance markers in two trails totalling 135 weaning pigs fed four 0.5% binder-aflatoxin diets and a purely aflatoxin contaminated diet. Results found that pigs fed with three of the four binder containing diets (sepiolite, calcium bentonite and HSCAS) had a noticeable higher ADG than pigs fed the aflatoxin only diet ($P<.05$). Only pigs fed the trial diet of sepiolite-aflatoxin had a distinctly improved gain:feed ratio ($P < .05$). Those individuals whose diets were supplemented with Na bentonite, HSCAS, sepiolite and zeolite all had performance markers similar to pigs fed the uncontaminated control diet (Schell et al., 1993). Cumulatively studies suggest that sepiolite addition results in a significant improvement in performance markers when added

to an aflatoxin contaminated diet. However, specific research into the potentially negative interaction between the binder and nutrients has yet to be fully investigated. The previously mentioned 2013 EFSA report deems sepiolite usage at 2% mix of feed ration as safe for a wide variety of species including poultry and pigs. This reports also highlights however that no such safety studies have been carried out with regard to use in ruminants.

### 2.2.7 Glucomannan

Esterified glucomannan is a high molecular-weight polymer whose mycotoxin binding ability is thought to be linked to an ion exchange. A previously mentioned study by Devreese et al also investigated the mycotoxin binding efficacy of gluco-mannan. Data showed reduced transepithelial passage of DON with addition of gluco-mannan binder from 37% to 57%. A study of mycotoxin adsorbents and their links to meat quality in broilers proved that addition of 0.05% esterified glucomannan helped to prevent the adverse effects of mycotoxins in a mould contaminated maize diet (Liu et al., 2011). Meat quality was assessed 45 minutes post-mortem using a chroma meter. Addition of esterified glucomannan in the case of 48 trialled birds significantly decreased the relative yellowness value of thigh muscle. However, this study also concluded that 0.05% EGM did not improve growth performance perhaps not consistent with other studies who may have used varying yeast formulations thus rendering results incomparable. This study also proved that treatment with mycotoxin only contaminated diet reduced retention of crude lipid and phosphorus. It was suggested that the injuries to the gastrointestinal tract caused by mycotoxins caused such deleterious effects on nutrient retention. A comparison of nutrient retention in feed containing binder was drawn. The addition of 0.05% EGM to the contaminated diets had no effect on apparent nutrient retention which suggests perhaps that EGM does not bind mycotoxin in the intestinal track.

### 2.2.8 Polymeric resins

Two of the most studied polymers are cholestyramine and polyvinylpyrrolidone also known as crospovidone. Ramos et al conducted in vitro adsorption tests of zearalenone by addition of a variety of binders; it was shown that cholestyramine was the best adsorbent, followed by crospovidone (Ramos et al., 1999b). Cholestyramine is an anion exchange resin with conflicting adsorption capabilities during in vivo and in vitro testing. In vitro binding capacity of cholestyramine for ochratoxin A and zearalenone were 9.6 mg/g respectively (Bauer, 1994). However, in vivo experiments within the same study suggested very limited

ability for binding and reduction of ochratoxin levels in blood, bile and tissues. Crospovidone is a highly polar amphoteric polymer only yet to have been studied in vitro and able to adsorb 313.7 µg zearalenone/g adsorbent (Ramos et al., 1999b). One study examined the use of two artificially synthesised co-polymers cross-linking methylene-bis-acrylamide (MBAA) and polyvinylpyrrolidone; CPVP1 and CPVP2 cryogels (Alegakis et al., 1999). This study evaluated the adsorption of zearalenone mycotoxin by both polymers. Adsorption values for each polymer were at 578.6 and 742.9 ug/ml respectively. The results suggested higher adsorption capacities than other tested binders as significant decrease in zearalenone concentrations were recorded, ranging from 33.5-66.2% per 25 mg of polymer.

# 3 Methods

To test the effects of the Mycotoxin binders, a modern stable with 96 pens was used. This stable is state of the art and was build 4 years ago. The 96 pens where filled with 14 pigs of Danish x Pietrain genotypes. This gave us a total of 1344 swine, which were distributed evenly among both sexes, giving us a total of 672 females and 672 castrated males. Evenly distributed to the 4 different groups, every group was made up of 168 male and 168 female animals, kept in 24 pens. Female and male pigs were kept in separate pens throughout the whole fattening period. All groups were equally distributed within the stable, as to prevent environmental issues to affect the outcome of the trial. Out of every group, 6 male and 6 female animals were randomly selected for blood sampling (48 in total). The animals selected were marked with an additional ear tag with an individual number, in order to relocate them at the time of the second sampling. The blood was collected from all animals via the vena cava cranialis. The samples were then immediately centrifuged to separate the serum, which was then frozen a few hours after collection. After the second sampling, all samples were sent to the Laboratory in one batch.

The animals were of about 32.1kg and 74 days, when introduced to the stable. They were to be slaughtered at 115-120kg, at around 160 days the earliest. Throughout the whole period, they were provided with water and feed ad libitum in 3 phases with 3 different diets made by the local mill. The basic diet also serves as the control diet for the control group. The parameters for the 3 feeds used can be found in table 1 below. All groups got the same feedstuff from the same silos. The diets were changed on day 34, and 62, on which days the bodyweights of every pen were noted as well. Therefore, the bodyweights measured display the individual phases of feeding.

*Table 1:Analysis of the feeds used*

| Parameters | | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|---|
| moisture | % | 12.43 | 12.20 | 12.00 |
| ash | % | 4.68 | 4.55 | 4.63 |
| CP | % | 16.40 | 15.30 | 14.23 |
| CL | % | 3.88 | 3.53 | 3.15 |
| CF | % | 3.75 | 3.95 | 4.18 |
| NFE | % | 58.88 | 60.48 | 61.83 |
| ME | MJ/kg | 13.05 | 13.18 | 12.95 |
| Lysin | % | 1.08 | 0.97 | 0.91 |

The basal diet was equal among all 4 groups, the only difference was the addition of 3 different Mycotoxin-Binders at a dosage of 2kg/1000kg of Feedstuff.

Treatment 1: Control diet plus B.I.O.Tox® Farm (0.2%)
Treatment 2: Control diet plus B.I.O.Tox® (0.2%)
Treatment 3; Control diet plus B.I.O.Tox® Activ8 (0.2%)
Control group: Control diet

The additives compositions were as follows:

B.I.O.Tox® Farm:

Composition: Attapulgite, Yeast Cell Wall, Vegetable Carbon, Bentonite (1m558), Bentonite (1m558i), Sepiolite, Magnesium oxide

B.I.O.Tox®:

Composition: Yeast Cell Wall, Vegetable Carbon, Bentonite (1m558), Bentonite (1m558i), Sepiolite

B.I.O.Tox® Activ8:

Composition: Vegetable Carbon, Bentonite (1m558), Sepiolite, phytogenic additives

Growth performance:

All pens were weighed 4 times throughout the period, once at the start of the trial, on day 34, on day 62 and when the first ones were sent for slaughter on day 83. The average daily gain was calculated and the averages for every pen where tested for their equality of means using SPSS. The results of this analysis can be found in the Annex.

Blood sampling:

On day 48 and 83, 48 blood samples were taken, evenly distributed among each group (12 per) and sex (6/6) as well as random selection within the stable regarding pen location.

These samples were analysed photometrically for Calcium, Iron, Copper, Magnesium and Zink. Manganese was measured via Atomic absorption spectroscopy. Vitamins A, D 25-OH and E where measured with a High Performance Liquid Chromatography (HPLC). All Measurements were performed by an independent laboratory. Afterwards, the results were analysed using the two-sided t-test for the equality of means using the Software SPSS.

# 4 Results

## 4.1 Weights

No statistical difference was proven for any of the weights and average daily gains (ADG) recorded throughout this trial. The weights were measured for every pen at the end of every part of the fattening process. In table 2, the weights of the different treatment groups as well as the control group can be seen at day1, end of phase 1 on day 33, end of phase 2 on day 61 and at the end of the trial on day 83. The significance for the equality of means between the three treatment groups and the control group can also be found in that table. The averages for the ADG during the different phases can be found in table 3, including the statistical significances for their equality of means. No significant difference was found in any of the treatments at any point.

*Table 2: Weights at the different points during the fattening period in kg.*

| | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| day 1 | Treatment 1 | 31.0833 | 2.83255 | 0.486 |
| | Treatment 2 | 30.9821 | 2.40141 | 0.379 |
| | Treatment 3 | 30.9167 | 3.33481 | 0.411 |
| | control group | 31.6667 | 2.91636 | |
| day 33 | Treatment 1 | 57.4398 | 4.33101 | 0.627 |
| | Treatment 2 | 57.2003 | 3.38957 | 0.454 |
| | Treatment 3 | 57.4977 | 4.01421 | 0.648 |
| | control group | 58.0549 | 4.38258 | |
| day 61 | Treatment 1 | 83.0964 | 5.22668 | 0.833 |
| | Treatment 2 | 82.8818 | 4.16657 | 0.700 |
| | Treatment 3 | 82.8633 | 4.62195 | 0.702 |
| | control group | 83.4208 | 5.38121 | |
| day 83 | Treatment 1 | 102.8496 | 5.90262 | 0.903 |
| | Treatment 2 | 101.3694 | 6.16374 | 0.441 |
| | Treatment 3 | 102.4017 | 5.49890 | 0.872 |
| | control group | 102.6529 | 5.23473 | |

*Table 3: Average daily gain for the different treatment in the different phases in kg*

| | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| day 1-33 | Treatment 1 | 0.7987 | 0.06638 | 0.957 |
| | Treatment 2 | 0.7945 | 0.05679 | 0.756 |
| | Treatment 3 | 0.8055 | 0.05042 | 0.709 |
| | control group | 0.7996 | 0.05724 | |
| day 33-61 | Treatment 1 | 0.9163 | 0.05620 | 0.520 |
| | Treatment 2 | 0.9172 | 0.05611 | 0.485 |
| | Treatment 3 | 0.9059 | 0.04314 | 1.000 |
| | control group | 0.9059 | 0.05487 | |
| day 61-83 | Treatment 1 | 0.9406 | 0.11986 | 0.478 |
| | Treatment 2 | 0.8804 | 0.24548 | 0.528 |
| | Treatment 3 | 0.9304 | 0.11951 | 0.675 |
| | control group | 0.9158 | 0.12033 | |

## 4.2 Calcium

*Table 4: Calcium in mmol/l - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 2.533 | 0.1231 | 0.231 |
| 18-Jun | Treatment 2 | 2.533 | 0.0985 | 0.181 |
| 18-Jun | Treatment 3 | 2.573 | 0.1272 | 0.704 |
| 18-Jun | control group | 2.592 | 0.1084 | |
| 23-Jul | Treatment 1 | 2.550 | 0.1000 | 0.052 |
| 23-Jul | Treatment 2 | 2.592 | 0.1311 | 0.304 |
| 23-Jul | Treatment 3 | 2.642 | 0.1379 | 0.885 |
| 23-Jul | control group | 2.650 | 0.1269 | |

If we compare the results for Calcium in Table 4, we see that almost all the results fall in a small range, leading to a small dispersal of results. This ranges from 2.3 mmol/l up to 2.8/2.9 mmol/l. As a result, the means of all groups are also grouped quite close to each other. Ranging from 2.533 (Treatment 1&2, 18$^{th}$ of June) to 2.650 in the control group on the 23$^{rd}$ of July. The standard deviations are minimal in all groups, the largest being 0.1379mmol/l in Treatment 3 on the 23$^{rd}$ of July. All groups concentration of Calcium in the blood rose between the two samples, but nothing out of the ordinary could be seen between the samples. None of the Treatment groups means showed significant differences to the control group, the only one coming close was Treatment 1 on the 23$^{rd}$ of July. There the mean of 2.550 mmol/l with a standard deviation of 0.100 mmol/l lead to an almost significant difference to the control group, which presented with a mean of 2.650 mmol/l and a range of 0.1269 mmol/l. The significance for the equality of means was p=0.052. The ranges for all the samples were also quite close together, all of them laying between 0.3 and 0.5, with the control group having a consistent range of 0.4 in both samples. Additionally, almost all values where in the physiological range of Calcium, which is given to be 2.4-3.5 mmol/l. The results of the statistical analysis, in which the means of the three treatment groups were compared to the mean of the control group, were negative, no other statistically significant differences between the means of either of the treatment groups and the control group could be found. The results can be found in Table 4. One outlier has been removed from analysis for Treatment 4 for the sampling date 18th June, this is due to the suspicion of the result

being incorrectly measured as it appears artificially low. Here 0.9 mmol was measured, but as it was inconsistent with any other values within this group, the closest data point being 2.3mmol/l and the value of the following sample collected from the same pig being 2.5 mmol/l, this was considered as an incorrect reading at sampling.

## 4.3 Iron

For Iron, it was quite similar to Calcium, the trend of the blood levels where rising between both samples. In every group, the mean of the second sample was higher than that of the first. The lowest mean in the first sample could be found with treatment 3, which was 16.925 µmol/l and with treatment 1 in the second sample at 20.425 µmol/l. The standard deviation for all samples and treatments were between 3.22 and 6.98 µmol/l. The highest measurements were both made in the samples of the control group on the 18$^{th}$ of June and on the 23$^{rd}$ of July. There are no apparent trends in either of the samples, none of them is especially low or high in both cases, they are all equally spread out. The ranges are not very consistent, with treatment 1 showing 10.6 µmol/l and 20.2 µmol/l, treatment 2 19.3 µmol/l and 23.4 µmol/l and treatment 3, 23.3 µmol/l and 9.9 µmol/l. The range for the control group was 16.90 µmol/l in the first sample and 22.5 µmol/l in the second sample. Even though the results were not as consistent as the data collected for Calcium, they were still all very similar and within the same ranges. And this is also shown by the t-test for the equality of means. None of the different treatments show a significant difference of means towards the control group, they are all quite similar, with p-values as high as 0.779, when comparing the sample of treatment 2 of the 23$^{rd}$ of July to the corresponding control group.

*Table 5: Iron in µmol/l - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 18.9583 | 3.69458 | 0.577 |
| 18-Jun | Treatment 2 | 17.358 | 6.2938 | 0.271 |
| 18-Jun | Treatment 3 | 16.925 | 5.9968 | 0.188 |
| 18-Jun | control group | 19.958 | 4.8866 | |
| 23-Jul | Treatment 1 | 20.425 | 6.0197 | 0.158 |
| 23-Jul | Treatment 2 | 23.650 | 6.8483 | 0.779 |
| 23-Jul | Treatment 3 | 20.900 | 3.2173 | 0.160 |
| 23-Jul | control group | 24.490 | 6.9834 | |

## 4.4 Copper

When looking at the results for copper in Table 6, we see that this time we don't have a trend as clear as with the previous minerals. Not all the treatment groups achieved higher results in the second than in the first sample. In Treatment 2, we see a decline of the mean from 25.475 µmol/l, the highest mean of the first sample date, to a mere 24.883 µmol/l, the lowest result in the second sample. The standard deviation stayed very similar though, at 4.497 µmol/l on the 23rd of July, compared to a standard deviation of 5.099 µmol/l in the first sample. But these deviations were also the largest on both sample dates. The other three groups showed an increase in blood Copper levels between the two samples, but they were all not big increases. Altogether, the Copper levels appear relatively stable over time, and all the means were well within the physiological range. If looking at the significances of the t-test for the equality of means between the control group and the 3 different treatments, it is also obvious, that there is no significant difference between the means. With p-values as high as 0.96, it is actually quite likely that there is quite a big link between the samples  The only noticeable thing is, that the standard deviation for the second sample of the control group appears quite small compared to that of the other groups at 1.9 µmol/l.

*Table 6: Copper in µmol/l - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 23.850 | 2.3357 | 0.799 |
| 18-Jun | Treatment 2 | 25.475 | 5.0983 | 0.449 |
| 18-Jun | Treatment 3 | 24.083 | 2.1557 | 0.958 |
| 18-Jun | control group | 24.142 | 3.1494 | |
| 23-Jul | Treatment 1 | 26.383 | 3.5754 | 0.343 |
| 23-Jul | Treatment 2 | 24.883 | 4.4974 | 0.858 |
| 23-Jul | Treatment 3 | 25.592 | 4.0913 | 0.749 |
| 23-Jul | control group | 25.160 | 1.9045 | |

## 4.5 Magnesium

The magnesium levels measured in all groups were below the normal physiological values. Therefore, I assumed that the feed fed to the animals during all phases did not contain enough Magnesium to cover the demands of the animals. When looking at the table, it is obvious, that the results are not going to give us much of a difference between the groups, as the range is quite small. The lowest mean for the both samples can be found with treatment 3. The mean for the first sample was at 0.817 mmol/l, while the result of the second sample was 0.850 mmol/l. The standard deviations or all samples are quite low, the largest deviation being found in Treatment 2 on the 23rd of July at 0.10 mmol/l. In all treatments and the control group, a positive trend can be seen, but not enough in any of them to reach the physiological range. The biggest improvement of Magnesium levels can be seen in the control group, the mean rose form 0.842 mmol/l to 0.920 mmol/l. This is an improvement of almost 0.08 mmol/l, more than any of the others. The second-best improvement was seen in treatment 1 (0.825 mmol/l to 0.883 mmol/l). If looking at the Significances, one value stands out, the significance for the equality of means between the control group and treatment 3 is quite small at p=0.070. But it is not a significant difference yet, therefore it is safe to say that there is no statistically significant difference between any of the means of the treatment group and the respective control group.

*Table 7: Magnesium in mmol/l - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 0.825 | 0.0622 | 0.534 |
| 18-Jun | Treatment 2 | 0.883 | 0.0937 | 0.223 |
| 18-Jun | Treatment 3 | 0.817 | 0.0718 | 0.387 |
| 18-Jun | control group | 0.842 | 0.0669 | |
| 23-Jul | Treatment 1 | 0.883 | 0.0577 | 0.223 |
| 23-Jul | Treatment 2 | 0.900 | 0.1044 | 0.624 |
| 23-Jul | Treatment 3 | 0.850 | 0.0905 | 0.070 |
| 23-Jul | control group | 0.920 | 0.0789 | |

## 4.6 Manganese

Like the others, the means regarding Manganese levels were quite equal, as seen in Table 8. The lowest mean in the first sample group was found with treatment 1, at 2.17 ng/ml, the highest one was measured with the control group, it was at 2.6. But the control group also presented with the highest standard deviation in the first sample group, being 0.216 ng/ml, whereas treatment one only had a standard deviation of 0.57 ng/ml. For the results of the 23rd of July, a different situation was seen. All means laid within 0.1 ng/ml to each other. The lowest one was Treatment 3 with 2.350 and the highest one Treatment 2 with 2.455 ng/ml. But again, the standard deviation was the largest in the treatment with the highest mean, being 1.17 ng/ml for Treatment 2. The smallest standard deviation was seen in the control group on the 23rd of July, being 0.22 ng/ml. One outlier was removed by me, for the second sample of the second treatment, one value was measured to be 15.4 ng/ml, more than 6 time as high as the mean of the control group and the other means. The other values appear to be quite similar again, the means of all the groups range between 2.17 ng/ml and 2.6 ng/ml. When looking at the results of the t-test, it is shown, that there were nor significant differences between any of the means, when compared to the respective control group.

*Table 8: Manganese in ng/ml - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 2.167 | 0.5726 | 0.140 |
| 18-Jun | Treatment 2 | 2.558 | 0.7255 | 0.894 |
| 18-Jun | Treatment 3 | 2.175 | 0.4434 | 0.120 |
| 18-Jun | control group | 2.600 | 0.7943 | |
| 23-Jul | Treatment 1 | 2.408 | 0.5384 | 0.962 |
| 23-Jul | Treatment 2 | 2.455 | 1.1733 | 0.887 |
| 23-Jul | Treatment 3 | 2.350 | 0.4543 | 0.753 |
| 23-Jul | control group | 2.400 | 0.2160 | |

## 4.7 Zinc

When looking at table 9, it is visible right away, that the results vary greatly. Both, the standard deviations and the means have quite large ranges. On both dates, the highest standard deviation was seen with Treatment 2, 8.92 µmol/l in the sample on the 18th of June, and 8.66 µmol/l in the sample of the 23rd of July. The highest mean for the 18th of June was also found with treatment 2 (18.1 µmol/l), the lowest one was with Treatment 3 at 13.3 µmol/l. For the second sample date, Treatment 1 presented with the highest mean between all the results, at 17.76 µmol/l. The range was relatively big though, it was 5.29 µmol/l. Treatment 2 was the only group showing a negative trend between the two samples, it dropped from 18.1 µmol/l to 16.3 µmol/l. If we now look at the statistical analysis, we see that in the first sample group on the 18th of June, the p-values were considerably lower than on the 23rd of July, meaning that the treatments were a lot more equal to the control group on the second date. P-Values as high as 0.902 can be seen here as a result of the t-test for equality of means between treatment 2 and the control group.

*Table 9: Zinc in µmol/l - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 14.350 | 1.7625 | 0.375 |
| 18-Jun | Treatment 2 | 18.100 | 8.9239 | 0.382 |
| 18-Jun | Treatment 3 | 13.317 | 2.2041 | 0.122 |
| 18-Jun | control group | 15.558 | 4.2262 | |
| 23-Jul | Treatment 1 | 17.758 | 5.2924 | 0.318 |
| 23-Jul | Treatment 2 | 16.300 | 8.6627 | 0.902 |
| 23-Jul | Treatment 3 | 15.842 | 2.0624 | 0.900 |
| 23-Jul | control group | 15.950 | 1.8710 | |

## 4.8 Vitamin D

When looking at the results for Vitamin D in Table 10, we notice that the Means appear to be quite widely distributed. This is due to the high levels in nmol/l, which range from 179.0 nmol/l for treatment 2 in the first sample to 248.09 nmol/l in treatment 2 on the 23rd of July. This also shows the positive trend that all the different groups displayed between the two samples. The standard deviations are quite similar to each other in both sample groups though, the first group had a small outlier though, Treatment one only displayed a standard deviation of 26.69 nmol/l. The Means on the 23rd of July were all between 227.48 nmol/l (Treatment 1) and 248.09 nmol/l (Treatment 2). The standard deviations ranged from 49.4 nmol/l to 59.87 nmol/l. In table 10, we can also see, that there is no evidence of any statistical differences in the means of the different groups. The lowest p-value was calculated for treatment 2 on the 18th of June, which was 0.265.

*Table 10: Vitamin D in nmol/l - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 219.0517 | 26.68924 | 0.366 |
| 18-Jun | Treatment 2 | 179.0300 | 42.63050 | 0.265 |
| 18-Jun | Treatment 3 | 198.2767 | 43.16558 | 0.843 |
| 18-Jun | control group | 202.3867 | 56.49690 | |
| 23-Jul | Treatment 1 | 227.4758 | 51.51014 | 0.469 |
| 23-Jul | Treatment 2 | 248.0883 | 59.87014 | 0.878 |
| 23-Jul | Treatment 3 | 227.9908 | 49.41762 | 0.473 |
| 23-Jul | control group | 244.2450 | 54.85323 | |

## 4.9 Vitamin A

This is the first group, in which we saw a definite decline between the means of the first and the second sample. In all groups, we noticed a drop by as much as 74 µg/l between the first and the second sample of Treatment 2 (409 µg/l to 335 µg/l). The control group went from 420 µg/l down to as little as 357.5 µg/l. Another interesting thing were the standard deviations, which were as big as 228.7 µg/l for Treatment 2 on the 18$^{th}$ of June. Closely followed by the control group on the same date with a deviation of just over 200 µg/l. The deviations were considerably smaller on the 23$^{rd}$ of July, with the highest one being seen in Treatment 3 with a standard deviation of 78.327. The means were also quite consistent on the 23$^{rd}$ of July, ranging from 331.58 µg/l to 357.50 µg/l. When looking at the Significance of the t-tests though, we see that, yet again, no statistically significant difference in the means of the different groups could be noted.

*Table 11: Vitamin A in µg/l - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 389.08 | 96.389 | 0.633 |
| 18-Jun | Treatment 2 | 409.18 | 228.741 | 0.903 |
| 18-Jun | Treatment 3 | 370.75 | 144.216 | 0.495 |
| 18-Jun | control group | 420.25 | 200.941 | |
| 23-Jul | Treatment 1 | 331.58 | 62.573 | 0.328 |
| 23-Jul | Treatment 2 | 335.42 | 36.405 | 0.287 |
| 23-Jul | Treatment 3 | 354.08 | 78.327 | 0.910 |
| 23-Jul | control group | 357.50 | 57.599 | |

## 4.10 Vitamin E

For Vitamin E, the means appear quite similar to each other and so do the standard deviations in both sample groups. The highest mean for the samples on the 18th of June was measured with Treatment 3, at 2.09 mg/l. The lowest for that same date was with Treatment 1 at 1.833 mg/l. The standard deviations were all between 0.53 and 0.63 mg/l. For the second sample group on the 23rd of July the means were almost all higher than they were on the 18th of June. The only one being lower is the control group, which went from 1.97 mg/l down to 1.85 mg/l. The standard deviations were a bit wider on the 23rd, reaching form 0.42 mg/l in the control group to 0.62 in Treatment 1. But Vitamin E still is the first group to present us with a statistically significant difference between the means of a treatment and the control group. There are two significant differences on the 23rd of July. Treatment 2 and 3 are both significantly higher than the control group on that day. The p values are 0.049 for the t-test for equality of means between Treatment 2 and the control group and 0.027 for Treatment 3.

*Table 12: Vitamin E in mg/l - Mean, Standard deviation and Significance of the t-test for equality of means of the treatments compared to the control group*

| date of sampling | Treatment | Mean | Std. Deviation | Sig. (2-sided) |
|---|---|---|---|---|
| 18-Jun | Treatment 1 | 1.833 | 0.6272 | 0.581 |
| 18-Jun | Treatment 2 | 1.873 | 0.6035 | 0.697 |
| 18-Jun | Treatment 3 | 2.092 | 0.5282 | 0.571 |
| 18-Jun | control group | 1.967 | 0.5365 | |
| 23-Jul | Treatment 1 | 2.100 | 0.6179 | 0.291 |
| 23-Jul | Treatment 2 | 2.258 | 0.4776 | 0.049 |
| 23-Jul | Treatment 3 | 2.350 | 0.5402 | 0.027 |
| 23-Jul | control group | 1.850 | 0.4197 | |

# 5 Discussion

Before discussing any further results, some things have to be noted: Between the two blood samples, taken on the 18th of June and the 23rd of July, two sampled animals from the control group either died during the course of experimentation or had to be euthanized. One individual suffered a broken leg therefore for welfare issues was euthanised, the other died as a result of gastric torsion. The pigs in question where from two different pens, in two different areas of the stable and no connection can be drawn between the two individuals, apart from being in the same experimental group. One of them was a female animal, the other one male, they were marked with ear tags number 5 and 12 respectively.

Furthermore, the results for levels of Vitamin E and Vitamin A could not be obtained for pig number 2, treatment group 1, as an insufficient amount of blood was collected for this individual to enable successful measurement of parameters.

Other than that, out of the 1344 pigs used in this trial, 40 either left the trial or died during the time of the trial. The majority of those that left the trial were separated as they had necrosis of the tail, in two cases they had an anal prolapse. The pigs that died mostly died due to gastric torsions, but unfortunately no records were kept concerning their pen of origin. Altogether, these 40 pigs represent a rate of 2.9% of the total group, which is a little high for a fattening program, but not too high regarding the environmental aspects at the time of this trial. Outside ambient temperatures of more than 40 degrees were reached on some days, temperatures that the ventilation system of the stables have trouble dealing with.

The results of this trial are perhaps as hoped, this is to say that data sampled shows no significance between the means of almost all groups and materials tested. If a correlation between binder vitamin interaction were to be expected, then we might expect significant difference between the means sampled serum levels for vitamins at the various sampling dates. Perhaps the majority of the data supports the null hypothesis that addition of mycotoxin binders does not affect vitamin and mineral serum levels. The only significance we found was a higher Vitamin E level in the sample from the second sampling day. If we are to assume no significant alterations in vitamin levels, we might not expect the difference regarding the means of the treatment groups for Vit E. However, it could also be suggested that the alteration in means values is not extreme and may be at tolerable levels. As a result,

it may be worth considering the practical impact of this data as it does not affect the animals negatively through low levels.

These results support the results published in the scientific opinion given by the European Food Safety Authority (EFSA) in 2013, that no negative effects were to be expected. However, it was not possible to reproduce the effects on final bodyweight obtained by both trials presented there. This is probably due to the significantly lower ratio of binders per kilogram used in this trial. This low a concentration was used, as this is in all cases the recommended amounts of the binders tested.

If one were to repeat this trial, higher doses might be worth testing, as well as changing the sensitivity of some of the tests for minerals. This was quite noticeable for Calcium for example. It would have been helpful to get the result in nmol/l as there must have been differences between the groups, but they were nor measurable in steps .1 mmol/l. The same was true for magnesium.

Altogether, these results are to be seen as a good result, as it shows, that these 3 commercially available mycotoxin binders can be used at their recommended dosages without causing any undesirable side effects.

# 6 Summary

The hypothesis of this thesis was, that there is no significant difference between the means the three groups fed with basal diets combined mycotoxin binders and a control group fed with nothing but the basal diet.

In this trial, 1344 pigs were fed 4 different rations, 3 of those with 3 different commercially available mycotoxin binders, at a dosage of 2kg/1000kg of basal diet, the control group was only given the basal diet. All feeds and water were available ad libitum at all times of the day. Blood samples were taken from 48 random pigs, equally distributed among all test groups and sex. The blood samples were repeated on the same animals 35 days later. When all samples were obtained, they were sent to an independent laboratory for analysis of the serum for levels of the following minerals and Vitamins: Calcium, Iron, Copper, Magnesium, Manganese, Zink, Vitamin D, Vitamin A and Vitamin E.

Only two statistically relevant results were obtained, both of them regarding Vitamin E. The pigs in treatment group 2, fed with 2kg/1000kg BioTox had a significantly higher mean than the control group. Their means were 2.258 mg/l for treatment 2 to 1.85 mg/l for the Control group. The mean of treatment 3 (B.I.O.Tox® Activ8) was even higher, at 2.35 mg/l. The significance for the difference between the third treatment and the control group was p=0.027.

No other differences in the equality of means were detectable.

This shows, that there is no danger of the mycotoxin binder to negatively affect pigs during the process of fattening if it is used in the recommended dosages.

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Thank you!

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