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EFFECT OF FEEDING A BLEND OF NATURALLY-CONTAMINATED CORN ON NUTRIENT DIGESTIBILITY AND FEED PREFERENCE IN WEANLING PIGS

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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at the University of Kentucky

By

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Lexington, Kentucky

2012

ABSTRACT OF THESIS

EFFECT OF FEEDING A BLEND OF NATURALLY-CONTAMINATED CORN ON NUTRIENT DIGESTIBILITY AND FEED PREFERENCE IN WEANLING PIGS

Two experiments were conducted to determine the effect of feeding diets with a 2009 and 2010 naturally-contaminated corn to weaning pigs. For both experiments three diets were blended to contain 100% 2010 naturally-contaminated corn (control), 50-50% blend of the 2009 naturally-contaminated corn and 2010 corn (Diet 2), and 100% 2009 naturally-contaminated corn (Diet 3). In Exp. 1, 24 crossbred pigs with an average body weight of 7.64 ± 0.70 kg were allotted to 4 replicates of 3 treatments with 2 pigs per pen, on the basis of gender, litter mate, and BW in a randomized complete block design. Fecal and urine samples were collected and dry matter, energy, and nitrogen apparent digestibility were determined. Dry matter, energy, and nitrogen digestibility were not affected by either Diet 3 or Diet 2 compared to the control diet. In Exp. 2, 30 crossbred pigs with an average body weight of 7.98 ± 1.15 kg were allotted to 3 replicates of 2 comparisons with 5 pigs per pen. Comparisons consisted of: 1) Control vs Diet 3, and 2) Control vs Diet 2. Two feeders were located in each pen containing one of the two diets. Feed preference and growth performance were determined. A preference for the feed containing 2010 corn feed was observed; pigs showed the ability to discriminate mycotoxin-contaminated feed (95.34 vs. 4.66%; $P< 0.01$). Nutrient digestibility was not affected by these diets, but a clear decrease in feed intake was observed in the pigs.

Key words: Digestibility, Mycotoxins, Naturally-contaminated Corn, Pigs, Preference

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This work is dedicated to my family, especially my parents, for all their support and love.

Este trabajo es dedicado a toda mi familia especialmente a mis padres por todo su apoyo y amor.

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CHAPTER 1. Introduction

In human history necessity inspires ingenious solutions. Different challenges have been appearing in terms of food acquisition by the human, from being nomads, to hunter-gatherer, until the beginning of a simple agricultural system when humans became sedentary. The population started increasing as did agricultural production; increasing crop products and this products storage was imminent. This way different mechanisms and technologies were developed to fight against bacteria, yeast, fungi and other microorganisms in order to preserve food through time. Processes such as drying, freezing, salting, sugaring, irradiation, burial in the ground, until current techniques such as artificial food additives, pulsed electric field processing, vacuum packing, high pressure food preservation and many others, are utilized on a regular basis.

Even with all these food preserving technologies it is difficult to mitigate the impact of harmful microorganisms on food/feed quality. There is not a single solution to the problem, and it is necessary to find small contributions in all the production segments in order to dissipate the problem. One of the challenges that modern agriculture industry faces is the appearance of fungi in a wide variety of substrates, including feed and foods. These fungi produce toxic metabolites called “mycotoxins”.

Mycotoxins were first mentioned in the early 1960s when the discovery of the aflatoxins was made (Richard, 2007). These metabolites are produced from mycotoxin-producing fungi such as *Aspergillus*, *Penicillium*, and *Fusarium*, and of which thousands are known to be toxic to animals and humans (Yiannikouris and Jouany, 2002). The most common classes of mycotoxins appear to be aflatoxins, trichothecenes, fumonisins, vomitoxin, zearalenone, ochratoxin A, and the ergot alkaloids that is known since the

middle ages (Hussein and Brasel, 2001). The implication of mycotoxins lies on the appearance of several toxicosis and diseases in both humans and animals, especially in monogastrics (Hussein and Brasel, 2001). Mycotoxins also affects prior, during, and post harvesting, transportation and storage of a wide variety of agricultural products, which is reflected in condemned agricultural products and important agro-economic losses (Zain, 2011).

The swine industry has experienced significant economic losses over the years due to feed containing mycotoxins (Vesonder and Hesseltine, 1981). The detrimental effects of mycotoxins in swine include chronic effects such us poor feed conversions, lower productivity, and immune suppression which decreases the resistance to infections (Grove et al., 1969). The severity of these affects are dependant on the type of mycotoxins consumed, the time of exposure, the way of inclusion, and the animal physiology stage. Young pigs are more susceptible than older pigs to damage from mycotoxins.

Finding solutions to this problem requires knowledge of the interactions of mycotoxins with animals. A considerable number of studies mention the effects of mycotoxins on feed intake, growth and reproductive performance, of which these are all types of clinical cases. But there is a lack of information in terms of how nutrient digestibility is affected by mycotoxins. Therefore, the objective of our study was to evaluate how nitrogen, energy, and dry matter digestibility was affected by a naturally-contaminated corn with vomitoxin, zearalenone, 15- acetyl DON and fumonisin B_1 in weaning pigs.

CHAPTER 2. Literature Review

2.1. Background

2.1.1. Mycotoxins and human health

Human exposure to mycotoxins can occur by ingestion, contact, and inhalation of food containing products with fungal growth. The effect of these toxins in human metabolism can be as simple as a non-detectable toxicosis to a potent acute and/or chronic intoxication case. Acute occurrences are not likely to occur in the United States, where the mitigation plans and controls for human consumption products are strict. But the greatest potential problem lies in a long-term exposure to low levels since where there are possible adverse effects.

Since aflatoxins were first found to be carcinogenic in the 1960s, there has been the isolation and characterization of thousands of fungal compounds (Jarvis and Miller, 2005), but few of them are known to be natural contaminants that impact to human health. Aflatoxins, ochratoxins, trichothecenes, zearalenone, and fumonisins are likely the mycotoxins with more implication in human health, and therefore, they are the most studied mycotoxins.

Aflatoxins

Aflatoxins and their effects on humans are well documented (Krishnamachari et al., 1975). The most common symptoms of aflatoxin ingestion are headache, nausea, vomiting, anorexia, gastrointestinal bleeding, abdominal pain, pulmonary and leg edema, fatty infiltration, necrosis in the liver, and in some cases death (Peraica et al., 1999).

Aflatoxin B_1 type is the most mentioned mycotoxin. This toxic metabolite has been associated with hepatocellular carcinoma (Linsell, 1980; Linsell and Peers, 1977; Shank, 1976; Shank and Wogan, 1964). In 1977 it was determined that aflatoxin B_1 was capable of binding to DNA, forming aflatoxin B_1-guanine attachments (Essigmann et al., 1977). More recent data provided strong evidence that aflatoxins are linked to mutations in the p53 gene, which is a tumor suppressor gene commonly mutated in people with cancer, implying that there is a G-T transversion in the gene. This discovery provided important information into the cause and development of carcinogenic tumors (Groopman et al., 1996).

Ochratoxin

When ochratoxin was discovered in the swine industry, pigs were presenting pain around the kidneys, drinking excessive water, urinating almost continuously, appearing depressed, and displaying a considerable decrease in feed intake (Hope and Hope, 2012) . In 1956 the first clinical case in humans was reported, with similar symptoms to swine, but its etiology was unknown. At that time the disease was called "Balkan endemic nephropathy" (Tanchev and Dorossiev, 1991). After the recognition of the toxic metabolite ochratoxin in food consumed in the Balkan countries, several investigations were conducted. These studies found the appearance of cancer in rats and mice, and also kidney tumors in patients with the "Balkan endemic nephropathy", providing important evidence that it was due to ochratoxin (Macgeorge and Mantle, 1990).

Ochratoxin is airborne in nature making its exposure a potential risk. A case of acute renal failure had been found in a woman that was exposed to a granary and grain

dust from contaminated wheat. Another case where analysis showed levels of ochratoxin above 1500 ppm in household dust collected from a house which residents experienced symptoms reminiscent of ochratoxin toxicosis in animals (Richard et al., 1999).

The most common symptoms of ochratoxin contamination in humans are anorexia, anemia, apoptosis, copper colored skin, fatigue, increased clotting time, increased eosinophils, increased leukocytes, increased reactive oxygen species and others symptoms (Chernozemsky et al., 1977; Müller et al., 1999; Schwerdt et al., 1999). And, as was mentioned before, it can be carcinogenic and can result in death.

Trichothecenes

Alimentary toxic aleukia (ATA), which occurred in Russia during 1944, is a disease characterized by the total atrophy of the bone marrow, agranulocytosis, necrotic angina, sepsis, hemorrhagic diathesis, and mortality reaching 80% (Joffe, 1986). Patients experienced vomiting, diarrhea, abdominal pain, and burning in the upper GI tract, followed by petechial hemorrhages that developed on skin, often accompanied by hemorrhages in the oral cavity, development of necrotic lesions, and enlargement of the local lymph node (Joffe, 1986). It was determined that this disease occurred when people ate overwintered cereal grains products. From a 20 year-storage grain, two fungi from the *Fusarium* specie were isolated and shown as responsible in producing trichothecenes such as T-2 toxin, neosolaniol, HT-2 toxin, and T-2 tetraol (Joffe, 1974; Schoental et al., 1979).

There is evidence that trichothecenes might cause Stachybotryotoxicoses disease in humans, but its most known to occur in horses and cattle. Dearborn et al. (1999) reported

some illness in people occupying buildings contaminated with *Stachybotrys*. These include pulmonary irritation, headaches, fatigue, malaise, and diarrhea (Croft et al., 1986). Dermatitis, inflammation of the nose, fever, chest pain, and leukemia are some other reported symptoms in humans handling contaminated hay (Robbins et al., 2000).

Deoxynivalenol, or DON, is one of the most common isolated mycotoxins from the trichothecenes group. Studies done in mice show an increase in immunoglobin A (IgA) levels in sera, with a similar diagnosis to the Glomerulonephritis disease in humans (Pestka et al., 1989). The common symptoms produced in humans include nausea, GI distress or pain, vomiting, diarrhea, headaches and throat irritation, and some patients had blood in their stools or developed a rash (Ueno, 1984).

Zearalenone

In Puerto Rico an uncommon case occurred in 7 and 8 year old children who were showing premature puberty with signs of premature thelarche, premature pubarche, prepubertal breast enlargement in boys and pseudopuberty in girls (Sáenz de Rodriguez et al., 1985). Investigators started to examine the local food, and found a high concentration of an estradiol-equivalent in some of the meat, leading them to consider all the estrogen-like substances used in cattle. They then found Ralgro®, a processed anabolic product made from zearalenone and approved for use in cattle and sheep in Puerto Rico. There have been suggestions that zearalenone can cause cervical cancer and premature thelarche, and it has also been shown that this mycotoxin binds to estrogen receptors of human myometrial tissue and can have lasting effects in the endocrine system (Szuets et al., 1997).

Fumonisins

In 1988, fumonisins, a class of mycotoxin produced by *F. verticillioides*, *F. proliferatum,* and at least one strain of *F. nygamai*, were discovered (Gelderblom et al., 1988). Fumonisin B_1 was the most studied and was shown to be responsible for leukoencephalomalacia in horses (Colvin and Harrison, 1992; Kellerman et al., 1990), pulmonary edema in swine, and liver cancer promoter and hepato- and nephrotoxicity in rats (Gelderblom et al., 1988). In humans there is not enough evidence to link the consumption of fumonisin, principally type B_1, to diseases such as esophageal cancer. It was found in southern Africa (Marasas, 1993), at the Linxian area of China (Li and Cheng, 1984) and more recently in northeastern Italy that the consumption of corn-containing fumonisins and esophageal cancer increased simultaneously (Franceschi et al., 1990), even though, fumonisin-exposed animals show that high doses after long periods of exposure triggers apoptosis mechanisms. Apoptosis is a key promoter in cancer and carcinogenesis induced by alterations in cellular sphingoid bases or sphingolipids (Voss et al., 1999). Also, it was found that fumonisin B_1 inhibited growth and induced morphological features consistent with apoptosis in human esophageal epithelial cell line and other human cells in-vitro (Tolleson et al., 1996). Sphingolipids are thought to play an important role in signal transmission and cell recognition, but sphinganine biosynthesis can be inhibited by fumonisin due to their close structural similarity (Wang et al., 1991).

2.1.2. Economic impacts of mycotoxins

The notable impact of mycotoxins in the feed and food production chain is that it affects each and every segment of it. Adverse economic effects can be due to differences in harvesting procedures, insecticide and fungicide use (which differs for various farm commodities), and also other factors that translate into low crops yields. The consequences are also found in the animal industry, generating immune suppression, decreasing growth rates and feed efficiency, ultimately resulting in low production and costly contingency programs. The appearance of fungus- toxic metabolites are known to be found pre, post, and during harvesting, storage, processing, transportation and even in animals' sub-products such as eggs, meat, and milk (Council for Agricultural Science and Technology, 2003). In addition, the implementation of technologies, research, strategies and programs to reduce mycotoxin appearance and risk, regulatory enforcement, mitigation, lawsuits, testing and quality control produce considerable costs for livestock and crop producers, and every entity involved.

Crops can be drastically battered by the influence of mycotoxins. The Food and Agriculture Organization (FAO) estimates that 25% to 50% of the world's food crops are affected by mycotoxins, resulting in the loss of over 1000 million tons per year of feedstuffs (Miller, 1995). The most affected crops in the Unites States are cottonseed, peanuts, and corn (Dorner, 2008; Snijders, 1990; Thiel et al., 1992), but economic losses have also been reported in wheat, sorghum, and other oilseeds (Park and Pohland, 1986). The import "refusal" of grains and grain products (ready to eat products) are a part of the economic impact, but in a small portion. The Food and Drug Administration (FDA) in

2001 only reported 4 cases from 1,781 refusals, with aflatoxin contamination, but, the high percentages of the economic impact lie on crop losses. A study done in the United States cites that there is a loss of $932,000,000 due to mycotoxin contamination, and $466,000,000 for regulatory enforcement, testing and other quality control measures annually (Council for Agricultural Science and Technology, 2003).

Table. 2.1. Adverse economic effects attributable to mycotoxins

Producer costs
- **Crops**
  - Yield losses
  - Restricted markets
  - Nonmarketable product
  - Price discounts
  - Increased production costs
    - Pest control
    - Irrigation
  - Increased postharvest costs
    - On-farm drying
    - On-farm testing and sampling
    - On-farm detoxification
  - Increased transportation costs
  - Inability to obtain loans on stored grain
  - Disposal of useless crops (buried, burning)
- **Livestock (beef, swine, poultry) producers**
  - Higher mortality rates
  - Reproductive failures
  - Reduced feed efficiency
    - Higher feed costs
    - Lower live weight
    - Infertility syndrome
    - Increased susceptibility to disease
  - Overall quality loss
  - Monitoring and testing
- **Dairy**
  - Higher mortality rates
  - Reproductive failures (abortions)
  - Reduced feed efficiency (as above)
  - Lower milk production
  - Nonmarketable milk
  - Monitoring and testing

Handler/Distributor costs
- Extra drying costs
- Excess storage capacity
- Losses In transit
- Loss of markets

Processor costs
- Milled corn products
  - Restricted markets
  - Product loss
- Peanut products
  - Insurance premiums
  - Restricted markets
  - Product loss
- Fermentation products

Consumer costs
- Less nutritious food
- Higher product prices
- Reduced Income due to lost work days from acute aflatoxicosis
- Long-term chronic effects from low-level contamination

Social costs
- Regulatory costs
  - Establishing standards and tolerances
  - Surveillance and assay
  - Enforcement
- Research and extension
- Education
- Lower foreign exchange earnings
- Increased costs of imports

Taken from CAST- Mycotoxins: Economic and Health Risks (1989)

Corn is the most widely produced grain in the United States, constituting more than 90 percent of the total production of feed grains (U.S. Department of Agriculture, 2010). Most corn is utilized as an ingredient in livestock feed, and the rest as starch, sweeteners, corn oil, industrial alcohol, fuel ethanol, and many other industrial products (U.S.

Department of Agriculture, 2010). Since 1970, economic impacts of mycotoxins in corn have been reported, but from 1970 until 1988, the incidence of mycotoxins was minimal. In 1973 a report showed low levels of contaminated corn with aflatoxins (1.7 to 2.3 ppb). Also it was reported that Midwestern corn had low levels of aflatoxins in the years 1964, 1965, and 1967 (Shotwell et al., 1973). In 1988, 9 states reported the presence of aflatoxins (*Wall Street Journal*, 1988). Also, more than 30% of the corn samples taken from Iowa and Illinois in this same year appeared to have concentrations of aflatoxins above 20 ppb, and 7.2% and 11.6 %, respectively, above 100 ppb (Hurburgh, 1989). In Mississippi, Louisiana, and Texas, the corn losses due to aflatoxins in 1998 were significantly high. Specifically in Mississippi, 20% of the 50 million bushel corn crop had aflatoxin levels of 20 to 150 ppb, and was sold at a discounted price. Another 4% was abandoned because its aflatoxin concentration was above 150 ppb (Robens and Cardwell, 2003).

Corn, like other grains, is often intended for animal feeding, and corn containing mycotoxins lead to economic losses due to higher mortality rates, reproductive failures, overall quality loss, monitoring, testing, and reduced feed efficiency. These effects ultimately lead to higher feed costs, lower live weight, infertility syndrome, and susceptibility to diseases (Placinta et al., 1999).

The impact of mycotoxins in ruminants is not as drastic as in monogastric animals, as their sensitivity for negative effects is lower. However, the production of milk, beef or wool, and their reproduction and growth can be altered, mainly when the consumption of contaminated feed is sustained for a prolonged period of time (Hussein and Brasel, 2001). The economic losses information of dairy producers in the United States is not available,

but there is an economic impact. Nevertheless, research has shown that aflatoxicosis results in low milk production, liver damage, weight loss, and reduced immune system function, (Bodine and Mertens, 1983) which translates into high production costs and lower income.

Poultry producers are also affected economically, with the most severe effects being seen in young birds. Although it takes high levels to cause mortality, feeding low levels of mycotoxins for prolonged periods of time can be detrimental. The first reported case of mycotoxins in poultry occurred in England in 1960, when 100,000 young turkeys died in the course of a few months, leading to an important economic impact for producers. They called this event the "Turkey X disease". Investigations were conducted to look for the cause and they found that a Brazilian peanut meal was highly toxic for turkeys, as well as for poultry and ducklings. Furthermore, they determined that this toxin was fungus-produced by *Aspergillus flavus*, and that is where the name for Aflatoxins came from: "A" from *Aspergillus* and "fla" from *flavus* (Heathcote and Hibbert, 1978). In 1983, Nichols reported that in the early 1970s, the losses due to aflatoxins were in excess of $100 million per year. Then, in 1984, Hesseltine et al. estimated losses of $143 million to the U.S. broiler industry.

Unlike in the cattle and poultry industries, the mycotoxin known as DON has a greater effect on swine. In 1981, Vesonder and Hesseltine mentioned some occurrences of this toxin in cereal grains, and also the financial disaster for many producers. As well, aflatoxins also generate an impact on hog p roducers where $100 million were the estimated losses in the southeastern United States in 1980, or on a verage, 10% of the value per hog in this area (Nichols, 1983). North Carolina and Georgia suffered losses of

$28 million and $22 million, respectively, with mortality rates of 23% (Nichols, 1983). According to findings in the 3 year experiment done by Wilson et al. (1984) on 54 herds in Georgia the most affected producers were the small ones (20 to 50 pigs), with mortality rates of 28% compared to 10% in herds with 200 or more animals. In the past several years the use of distillers dried grains with solubles (DDGS) have been increasing significantly and animals that are the consumers can be fed with higher levels of mycotoxins because the concentration in DDGS can be up to three times more when compared to grains (Wu and Munkvold, 2008). A study was performed by Wu et al. (2008) in order to develop livestock models and estimate current losses in the swine industry. The results of this study estimated losses from $2-$18 million annually in weight gain reduction due to fumonisins in feed containing DDGS in 2006.

It is obvious that mycotoxins in crops and livestock, as well as the mitigation plan costs which are very high, clearly impose a significant economic problem in the United States and the worldwide economy. Table 2.2 shows an estimate of the money these three sectors can lose annually due to the major mycotoxin contaminants (aflatoxins, fumonisins and deoxynivalenol) in the United States. There are some suggestions of how to reduce mycotoxin impacts, but still, there is a lot of work that needs to be done. Mycotoxins are a tough enemy and perhaps we will have to deal with them always, but the need for more solutions remains if we are to avoid future crises.

Table 2.2 Potential total economic costs of Mycotoxins (in millions of dollars).[1]

Item	5th percentile	Median	Mean	95th percentile
Crop costs	418	882	932	1,656
Mitigation costs[2]	209	441	466	828
Livestock costs	2	6	6	12
Total	629	1,329	1,404	2,496

[1] Taken from CAST (2003).

[2] Mitigation costs include: Developing fungus-resistant grains, altered farming practices, applying additional insecticides, fungicides and fertilizer, improved handling after the harvest, improved storage and transportation conditions, more efficient drying, additional quality control procedures to monitor moisture and toxins, and amelioration of contaminated grains through physical and chemical treatments.

2.2. Mycotoxins overview

2.2.1. Major mycotoxins

During the period from 1800 to 1900 a concept called "secondary metabolite" was becoming popular among researchers worldwide. This concept affirmed that organisms such as fungi and microbes produce compounds that are not utilized by their metabolism, but are often, and usually, incorporated into the medium that surrounds them. Later on some of these secondary metabolites were known to have benefits in humans and were called "antibiotics" when used them in treating diseases and entered into the market. At the same time it was seen that not all of them were beneficial, but that some can be harmful and toxic to animal species. Therefore, in 1955 the term "mycotoxicoses" was introduced (Forgacs et al., 1955).

The function of producing mycotoxins by the fungus has not been conclusively established, but it is believed that they might play a role in eliminating competition from their environment, by creating a non-reliable medium for other microorganisms (Brase et al., 2009). The number of mycotoxins that exist is extremely hard to determine. Some estimates have been done over the years and in 1978 researchers catalogued as potential mycotoxins 500 fungus species which produced around 1,200 secondary fungal

metabolites (Turner, 1978). By 1983, 2,000 more metabolites with potential mycotoxin characteristics were catalogued, which were produced by 1,100 species (Turner and Alderidge, 1983). And in 2006 a more conservative result was affirmed saying that more than 300 mycotoxins were known by this time (Akande et al., 2006). Nevertheless, researchers have focused on five groups of known mycotoxins because of their toxicity in humans and animals (aflatoxins, ochratoxin, trichothecenes, zearalenone and fumonisin), and because of the significant health and economic impacts these generates.

Both fungal growth and mycotoxin production are dependent on environmental factors, with the limits for mycotoxin production usually being narrower than those for growth only (Bennett and Klich, 2003). There are different factors and their interactions play important roles in either increasing and decreasing growth or mycotoxin production. Table 2.3 presents the most relevant factors known to influence mycotoxin production.

Table 2.3. Environmental factors influencing mycotoxin production.[1]

Physical factors	Chemical factors	Biological factors
Temperature	Atmosphere	Fungal plant pathogens
Water content	Substrate composition	Microbial competition
Mechanical damage	pH	
Time/season	Fungicides	

[1] Adapted from Silva et al. (1998).

Temperature: Mycotoxin production is very dependent on temperature and water activity. Mycotoxin production usually occurs at the same temperatures needed for optimal growth. As examples, *Penicillium* produces mycotoxins at lower temperatures than *Aspergillus*, and at 5° C *Aspergillus* cannot produce aflatoxins and ochratoxin, whereas *Penicillium* and *Fusarium* are able to produce mycotoxins (Bullerman et al., 1984; Silva et al., 1998).

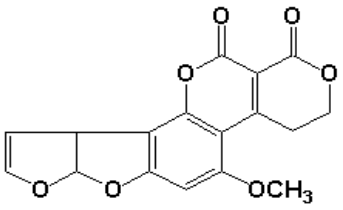
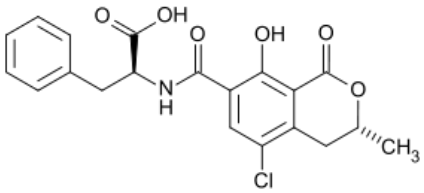
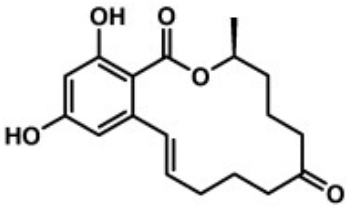
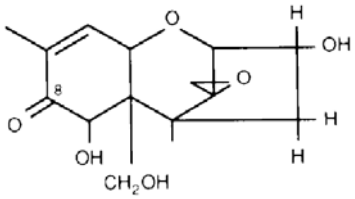
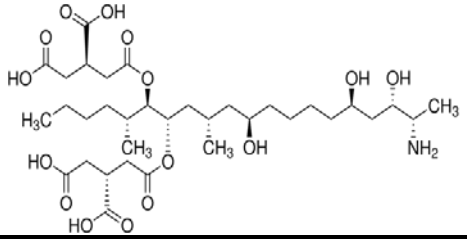
Water content: The term used to describe water content is water activity (a_w), because this does not include the bound water which is not available for the fungi. Most food borne fungi grow at minimal a_w of 0.8, which is lower than the a_w needed for bacterial growth (0.9). The optimal a_w for molds are usually close to 1 (Silva et al., 1998). Mycotoxins occur at higher water contents than needed for growth.

pH: Mycotoxin production usually takes place at a different pH optimum than fungal growth. Most food borne fungi can grow from pH 2.5 to pH 9.5, with an optimal pH from 4.5 to 6.5.

Substrate composition: Fungi can be very specific to a certain composition of the substrate. For example, *Penicillium crustosum*, *P. commune* and *P. echinulatum* are common only on nuts and other lipid- and protein-rich substrates like meat and cheese. Since fungi are heterotroph organisms, they need an organic source like glucose, maltose, saccharose and other water-soluble carbohydrates (Samson and Reenen-Hoekstra, 1988).

Table 2.4 shows the most common fungi species which metabolize these 5 groups of mycotoxins. Depending on their chemical structure, the biological effect in the affected organism is different, and it can be carcinogenic, teratogenic, mutagenic, estrogenic, neurotoxic or immunotoxic (Yiannikouris and Jouany, 2002a).

Table 2.4. Fungi and their associated mycotoxins.[1]

Fungi	Mycotoxins
Aspergillus flavus, A. parasiticus, A. nomius	Aflatoxins (B1): (B2, G1, G2, M1, and M2) 
Penicillium verrucosum, Aspergillus clavatus	Ochratoxin A: 
Fusarium graminearum, F. culmorum, F. crookwellense	Zearalenone: 
Fusarium sporotrichioides, F. graminearum, F. culmorum, F. poae, F. roseum, F. tricinctum, F. acuminatum	Trichothecenes (Deoxynivalenol): 
Fusarium moniliforme, F. proliferatum	Fumonisins B1: (B2, B3) 

[1] Adapted from Yiannikouris and Jouany (2002).

To combat against the harmful actions mycotoxins cause to animal welfare, it is necessary to understand the diversity of biochemical and cellular mechanisms of toxicity and, in that way, comprehend how toxic compounds alter the normal behavior of the

molecules in living organisms. Table 2.5 shows a summary of how some mycotoxins of interest interact with cells and initiate the toxicity cascade of events.

Table 2.5. Summary of the probable primary biochemical lesions and cascade of events of some important mycotoxins[1]

Mycotoxin	**Cascade of events**
Aflatoxin (Eaton and Gallagher, 1994)	Metabolic activation → DNA modification → cell deregulation → cell death/transformation
Deoxynivalenol (Rotter et al., 1996a)	Inhibition of protein synthesis → disruption of cytokine regulation → altered cell proliferation → cell death/apoptosis
Fumonisins (Riley et al., 1996)	Sphinganine N-acetyl →disrupted lipid metabolism → cell deregulation → cell death/apoptosis
Zearalenone (McLachlan, 1993)	Cytosolic estrogen receptor → estrogenic response → disruption of hormonal control → ?[2]

[1] Adapted from (Riley, 1998)
[2] Not enough evidence to conclude posterior events.

Aflatoxins

Aflatoxins are not only well known for their capability of producing important biological impacts on human and animal health but also for their economic impact. This group of mycotoxins is highly toxic and they belong to a group of difuranocumarinic derivatives that are structurally related (Mejía et al., 2011). A total of 18 types have been identified at the present, with the most frequent being B_1, B_2, G_1, G_2, M_1, and M_2, produced by fungus of genus *Aspergillus* spp. (Figure 2.2.). Aflatoxins B_2 and G_2 are relatively non-toxic unless they are metabolically oxidized into B_1 and G_1 in vivo (Kensler et al., 2011). The designation of the letter B resulted from the exhibition by these compounds of blue (B) fluorescence under UV-light and they are characterized by a cyclopentenone ring to the lactone of the coumarin moiety ring. The designation G came from yellow-green fluorescence which contains a fused lactone ring. And the M

designation, which are hydroxylated derivates from B_1 and B_2, come from their appearance in milk (M) and meat (Kensler et al., 2011).

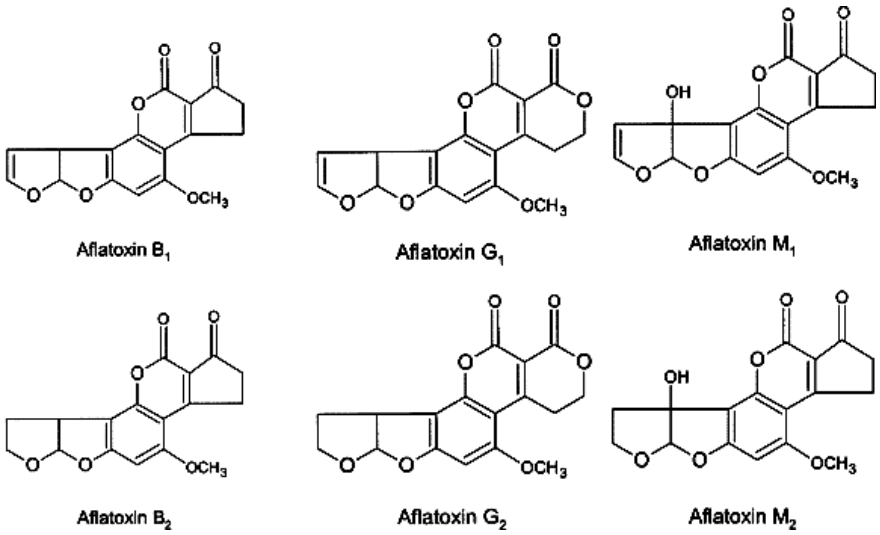


Figure 2.1 Common aflatoxin structures (Zain, 2011).

Aflatoxin B_1 is the most toxic of all the known types, and it is associated with immune suppression and liver cancer. When the exposure to B_1 is in high quantities (> 6000 mg/day), it can cause acute toxicity with lethal effects but when the exposure is in small doses for prolonged periods, it is carcinogenic (Groopman and Kensler, 1999). It has been classified as Group I carcinogenic in humans by the International Agency for Research on Cancer (IARC). Aflatoxin B_1 has been found in many crops including cotton, maize, nuts, peanuts, wheat, rice barley, and others. Its incidence depends on environmental factors like the substrate, temperature, pH, humidity, and other fungi in order to grow. The medium has to be adequate for their normal development (Bhatnagar et al., 2002). When the conidia (spores) encounter a nutrient source with favorable conditions, the fungus colonizes and produces aflatoxins (Payne, 1992). It can be found in its conjugated form, as a soluble or "masked mycotoxins", or incorporated/associated

with macromolecules called “bound mycotoxins”. Masked or bound mycotoxins can appear after being metabolized by living plants, fungi and mammals, or also after food processing (Mejía et al., 2011).

Aflatoxin B_1 is metabolized by the liver into 8, 9-epoxide, which is a highly reactive chemical compound due to its unsaturated bond at the 8, 9 position of the terminal furan ring (Groopman and Kensler, 2005). Following its formation, this 8, 9-epoxide compound can bind to proteins, DNA, and other important cellular compounds, forming adducts which interrupts the normal function of the cell. In the case of the DNA, it can lead to a loss of control over cellular growth and division (Groopman and Kensler, 2005). Nevertheless, humans and animals have mechanisms to correct DNA damage caused by the 8, 9-epoxide. For example, glutathione S-transferase mediates the reaction of 8, 9-epoxide to the endogenous compound glutathione that is not toxic. It was shown by Johnson et al. in 1997 that animals which are less susceptible to carcinogenic effects of aflatoxins, such as mice, show three to five times more glutathione S-transferase activity compared to more susceptible animals like rats. Humans have less glutathione S-transferase activity than mice and rats, suggesting that humans are less capable of detoxifying this metabolite (Johnson et al., 1997).

The biosynthesis of aflatoxins B_1 and B_2 has been well studied, and it consists of at least 16 steps before the first stable intermediate, norsolorinic acid (NA) (Figure 2.3). As with many other economically important fungi, the sexual stage of A. *flavus* and *A. parasiticus* is unknown, but they can undergo genetic recombination parasexually (Papa, 1973). It is known that the production of the secondary metabolites (idiophase) occurs right after the growth phase of the culture (trophophase) has slowed.

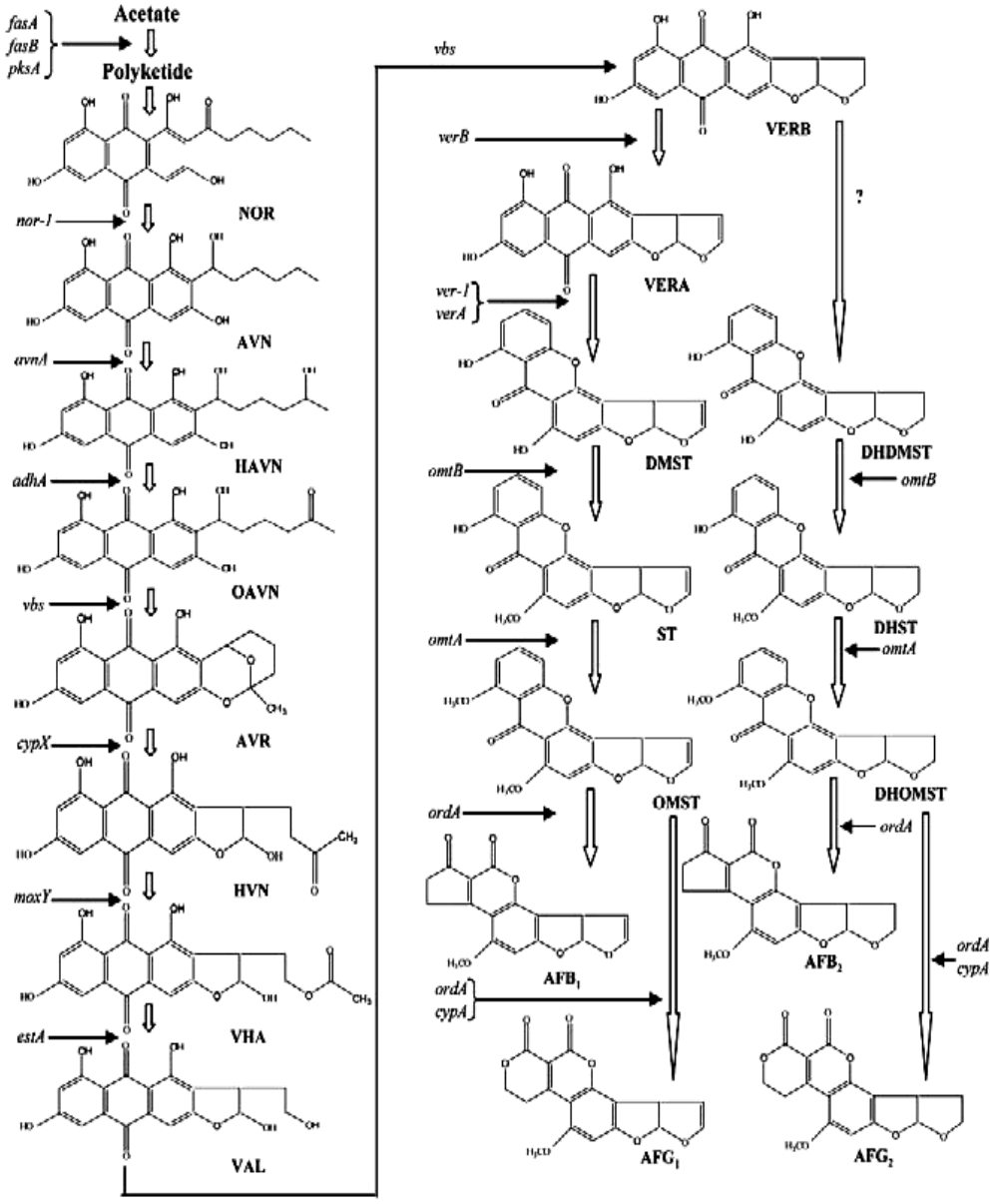


Figure 2.2. Aflatoxin biosynthesis pathway (Do and Choi, 2007). Abbreviations: NOR, norsolorinic acid; AVN, averantin; HAVN, 5- hydroxyaverantin; OAVN, oxoaverantin; AVR, averufin; VHA, versiconal hemiacetal acetate; VAL, versiconal; VERB, versicolorin B; VERA, versicolorin A; DMST, demethylsterigmatocystin; DHDMST, dihdrodemethylsterigmatocystin; ST, sterigmatocystin; DHST, dihydrosterigmatocystin; OMST, l-methylsterigmatocystin; DHOMST, dihydro-l methylsterigmatocystin.

Trichothecenes

Interest in trichothecenes was generated due to their constant contamination of human food and animal feed resulting in a worldwide problem. It has been well documented that they are nonspecific in their host, and that they inhibit protein synthesis in a wide range of eukaryotic organisms including animals, fungi, and plants (Cundliff et al., 1974), thereby impairing human and animal health. Like other fungal secondary metabolites, the production of this mycotoxin is not essential for fungi performance and reproduction (McCormick et al., 2011).

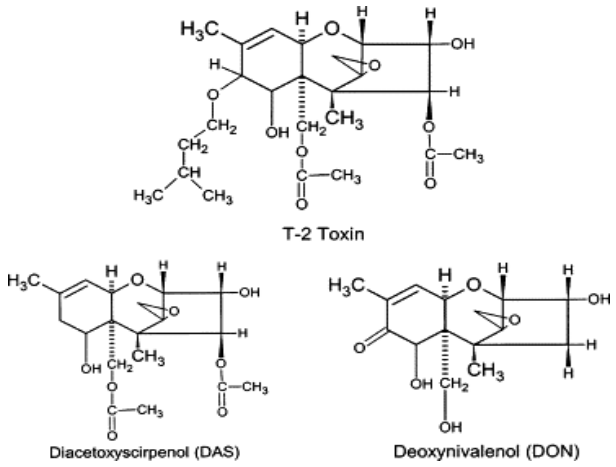


Figure 2.3. Structure of T-2 toxin, diacetoxyscirpenol, and deoxynivalenol (DON) (Mohamed, 2011).

Nowadays the total number of natural-occurring trichothecenes exceeds 60. All of them share a tricyclic nucleus name trichothecene (Figure 2.4) and contain an epoxide at C-12 and C-13, which gives it its toxic characteristics. The chemical differences between the trichothecenes metabolites vary in both the position and number of hydroxylations, as well in the position number and complexity of esterification (Bamburg, 1976). The *Fusarium*-produced trichothecenes are the most studied. Six of them have been well documented including *F. sporotrichioides* and *F. poae* that produce mainly T-2 toxin and

F.crookwellense, F. culmorum, F. graminearum, and *F. sambucinum*, which produce mainly diacetoxyscirpenol and deoxynivalenol (Figure 2.4) (Lauren et al., 1987).

Trichothecenes biosynthesis proceed from Trichodiene, a natural product first isolated from *F. roseum*, and involves a sequence of oxygenations, isomerizations, cyclizations and esterifications, requiring 10 steps for deoxynivalenol formation, 12 steps for diacetoxyscirpenol, and 14 steps for T-2 toxin, which is the most complex metabolite (Figure 2.5).

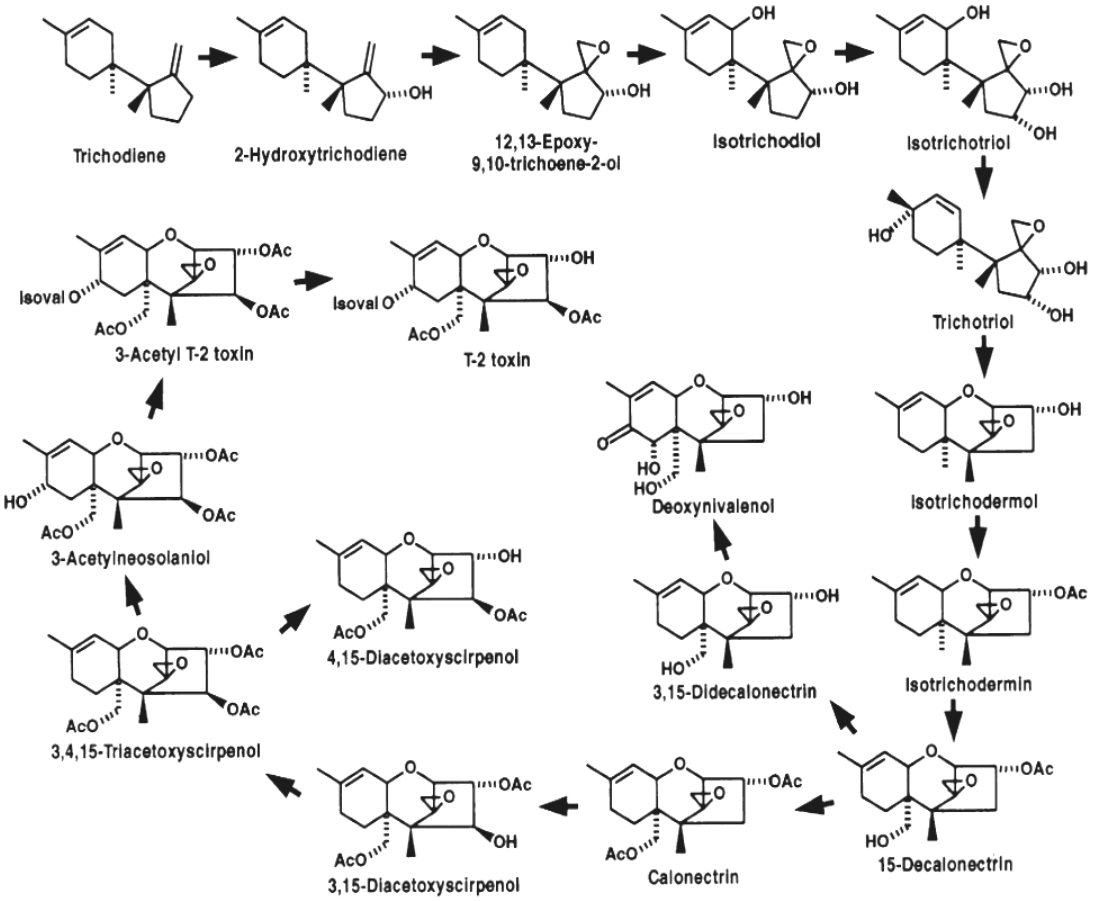
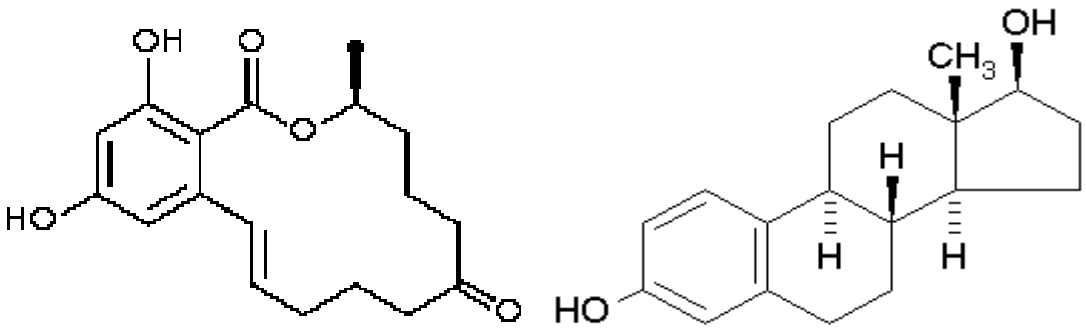


Figure 2.4. Trichothecene biosynthesis pathway in fusarium species (Desjardins et al., 1993).

Zearalenone

Zearalenone is a 1 4-membered orsellinic acid type macrolide, biosynthesized through a polyketide pathway in various *Fusarium* fungi, such as *graminearum*, *culmorum*, *equiseti* and *crookwellense* which colonize corn, barley, oats, wheat, and other grains (Bennett and Klich, 2003). It was first isolated from the mycelium of the fungus *Gibberella zeae* (*Fusarium graminearum*) and is now considered the progenitor of the family "resorcylic acid lactones" (RALs) found in nature: for example, hypothemycin, monorden and monocillin (Winssinger and Barluenga, 2007). Other important related metabolites such as α–zearalenol and β–zearalenol, can be produced by these fungi in small amounts, (Richardson et al., 1985). All zearalenones are non-steroidal estrogenic compounds, but their similarities with the estrogen chemical structure (Figure 2.6) makes them mimic natural reactions in animal and human metabolism. This is true despite the fact that α–zearalenol has a h igher estrogenic potential than both zearalenone and β–zearalenol (Hagler et al., 1979; Peters, 1972), probably due to its binding affinity to estrogen receptors (receptors for estradiol-17β) located in the uterus, liver, mammary gland, and hypothalamus (Fitzpatrick et al., 1989).



Zearalenone Estrogen

Figure 2.5. Chemical structures of zearalenone and estrogen (Gray et al., 2004).

Zearalenone is biotransformed mainly in the liver to α – and β –zearalenol in ratios varying between animals species (Zinedine et al., 2007). In the case of pigs, they mainly transform zearalenone to the more potent α –zearalenol, explaining their sensitivity to this secondary metabolite (Malekinejad et al., 2006).

Fumonisins

Fumonisins are primarily produced by s ome members of the fungi *Gibberella fujikuroi* species complex with *F. proliferatum* and *F. verticillioides* as the chief producer (Leslie et al., 1992). Fumonisins are a group of polyketide-derived mycotoxin, known to contain an 18-carbon backbone with varying side-groups, and classified into five series A, B, C, P (Bartok et al., 2006), and a new series recently identified, partially hydrolyzed B (PHFB) (Bartok et al., 2008). B-series fumonisin are the most abundant, and fumonisin B_1 is the most toxic analogue and is the most commonly found in naturally-contaminated corn (Marasas, 2001; Nelson et al., 1993).

Fumonisins are known to be structurally similar to the sphingoid base backbone of the sphingolipids, and that is why there are suggestions that both may be biosynthetically related (Figure 2.7); (Plattner and Shackelford, 1992). An understanding of the complete biosynthesis of fumonisins can be very helpful in understanding how to prevent their toxicity. Fumonisin inhibits ceramide synthase, causing accumulation of bioactive intermediates of sphingolipid metabolism including sphinganine, other sphingoid bases, and derivatives, but also reducing complex sphingolipids, which interfere with the normal function of some membrane proteins (Marasas et al., 2004). The accumulation of these sphingoid bases is the primary cause of the toxicity of fumonisin B_1 (Merrill et al., 2001).

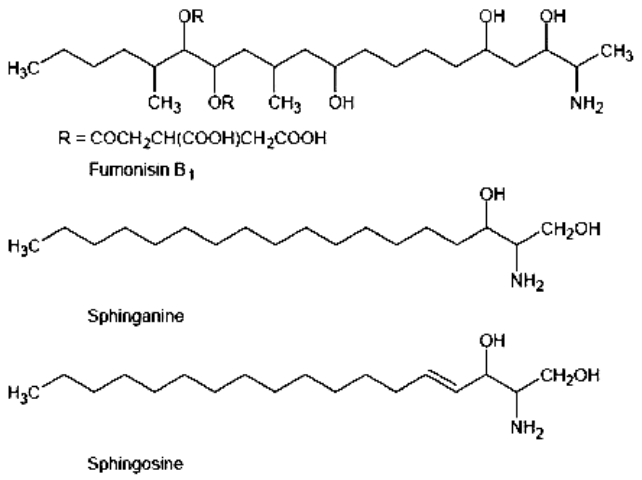


Figure 2.6. Structure of fumonisin B_1, sphinganine and sphingosine (Wang et al., 1991)

2.2.2. Mycotoxin interactions

Often times in raw materials and animal feeds more than one mycotoxin-producing mold appears. It is common for animals to present symptoms that cannot be explained by the levels of an individual mycotoxin, indicating that there are interactions between different mycotoxins. Depending on t he environmental conditions, there are several combinations of mycotoxins that frequently occur (Speijers and Speijers, 2004), and their interaction can result in synergistic, additive, and/or antagonistic effects (Figure 2.8).

Livestock are exposed to a complex mixture of mycotoxins. Sometimes the evaluation of each mycotoxin in the feed does not explain the consequences seen in the field. When the combined effect of two mycotoxins is much greater than the individual effect of each toxin by itself, it is called a synergistic effect (example: 2 + 2 = > 4). When the effect produced by two or more mycotoxins is equal to the sum of their separate toxic potential, it is called an additive effect (example: 2 + 2 = 4). A more rare effect is when

the predicted response is less from each toxin individually, called antagonistic effect (example: 2 + 2 = < 4) (Speijers and Speijers, 2004).

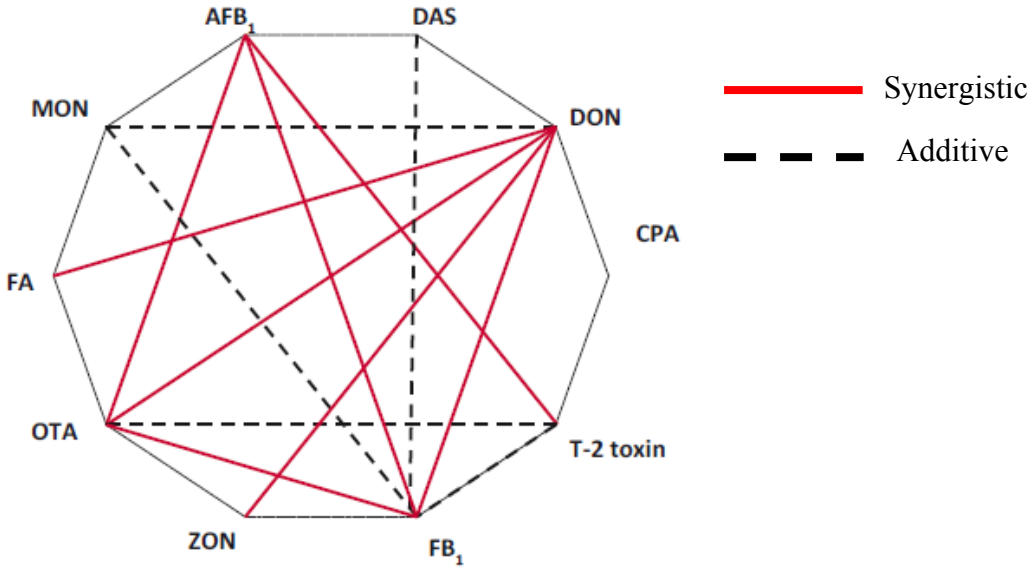


Figure 2.7. Mycotoxin interactions in swine (Borutova and Pedrosa, 2011). Abbreviations: AFB1, Aflatoxin B_1; FB1, Fumonisin B_1; DON, Deoxynivalenol; OTA, Ochratoxin A; ZON, Zearalenone; FA, Fusaric acid; DAS, Diacetoxyscirpenol; CPA, Cyclopiazonic acid; MON, Moniliformin.

The major concern in pigs is focused on the interaction between deoxynivalenol and fusaric acid (DON-FA) (D'Mello et al., 1999; Raymond et al., 2005), deoxynivalenol and fumonisin B_1 (DON-FB_1), aflatoxin and ochratoxin (AF-OTA) as well as aflatoxin and T-2 (AF-T-2) (D'Mello et al., 1999; Huff et al., 1988). Several studies have shown the synergistic reaction between mycotoxins. A 2004 study showed sudden death in piglets fed diets for several days with concentrations ranging from 10 to 40 ppm of fumonisin B_1 and 20 to 39 ppm of ochratoxin. The pigs presented pathological signs of both toxins such as pulmonary edema, kidney lesion, and liver lesion (Creppy et al., 2004). The same year another experiment showed suppression of both radical and antibody formation only

after the combination of ochratoxin and FB_1 or deoxynivalenol, which did not occur when ochratoxin was administered alone (Speijers and Speijers, 2004). Later on in 2009, Zielonka et al. reported the difficult examination of histopathological lesions caused by deoxynivalenol intoxication because of the common, often synergistic reaction of this mycotoxin with other toxins such as zearalenone. As is shown in Table 2.6, more studies have been conducted, but not enough to have a clear understanding of both additive and synergistic effects of mycotoxins.

Table 2.6. Mycotoxin interactions in swine[1,2]

Mycotoxin	Species tested	Effect	Reference
AFB_1 + OTA	Pigs	Synergistic	(D'Mello et al., 1999; Huff et al., 1988)
AFB_1 + FB_1	Growing pigs	Synergistic	(Harvey et al., 1995)
AFB_1 + FB_1	Pigs	Synergistic	(Liu et al., 2002)
AFB_1 + T_2	Pigs	Synergistic	(D'Mello et al., 1999)
DON + FA	Pigs	Synergistic	(D'Mello et al., 1999; Raymond et al., 2005)
MON + FB_1	Pigs	Additive	(D'Mello et al., 1999)
MON + DON	Pigs	Additive	(D'Mello et al., 1999)
OTA + DON	Weaned piglets	Synergistic	(Speijers and Speijers, 2004)
OTA + FB_1	Weaned piglets, piglets	Synergistic	(Creppy et al., 2004; Speijers and Speijers, 2004)
OTA + T_2	Weaned piglets	Additive	(Speijers and Speijers, 2004)
DON + ZON	Pigs	Synergistic	(Zielonka et al., 2009)
FB_1 + DAS	Pigs	Additive	(D'Mello et al., 1999)
FB_1 + DON	Pigs	Synergistic	(D'Mello et al., 1999; Huff et al., 1988; Speijers and Speijers, 2004)

[1] Adapted from Borutova and Pedrosa, 2011
[2] Abbreviations: AFB_1 – Aflatoxin B_1; FB_1 – Fumonisin B_1; DON – Deoxynivalenol; OTA – Ochratoxin A; ZON – Zearalenone; FA – Fusaric acid; DAS – Diacetoxyscirpenol; MON – Moniliformin

There is a lack of information about mycotoxin interactions, and definitely it is a topic that needs to be taken into account and given the attention it deserves. The fact that fungus from the same species can produce several mycotoxins, the fact that more than one fungus is usually infecting multiple grains and commodities, and the fact that all of these are mixed together and used in animal feeds; reiterates the importance of mycotoxin interactions in animal production.

2.2.3 Mycotoxins and feed intake

Many studies have shown that the consumption of diets containing mycotoxins by pigs have a drastic effect in feed intake and growth performance. In the case of DON, reduced feed intake is the principal effect seen in pigs (Dorner, 2008; Friend et al., 1992). Table 2.7 presents several studies showing the effects of different concentrations of DON on feed intake, where concentrations from 1 ppm start showing a decrease in feed consumption in growing pigs, followed by complete refusal when it reaches 12 ppm in the diet (Young et al., 1983).

Early studies indicate that DON is a potent feed intake inhibitor. In quantitative terms, marked effects of DON on feed intake have been observed particularly in the range from 6 - 15 ppm in the diet, as is shown in Figure 2.9 where feed intake was only 38% of the control diet in diets containing 15 ppm (Trenholm et al., 1994). Nevertheless, a particular feature observed with DON is that appetite depression effect can be immediate, and some recovery can occur over time without withdrawal of DON from the feed. Thus, it was noted in one study that partial, dose-dependent, adaptation to DON-contaminated diets occurs, with the effects being reflected in proportionate reductions in weight gain (Trenholm et al., 1994). Another study observed a reduction in feed intake for two days of feeding the contaminated diet followed by sufficient compensation thereafter to permit feed intakes and growth rates equivalent to those in control pigs (Prelusky et al., 1994).

Table 2.7. Summary of selected references on deoxynivalenol (DON) and feed intake (FI) in swine.[1]

Source of DON[2]	DON, ppm	ZEA, ppm	Sex	Age or BW	Effect[3]	Reference
Purified	3.6	1	Not stated	20-24 Kg	↓FI 20%	(Forsyth et al., 1977)
	7.2	1			↓FI 44%	
	40	1			↓FI 90% Vomiting	
Natural contamination	1.3	0	Not stated	3 wk	↓ADG ↓FE	(Young et al., 1983)
	12	0.2			Almost 100% feed refusal	
	20	0.9			Vomiting	
Natural contamination	0.75-2	0.03-0.28	Barrows, gilts	3-7 wk	↓FI	(Trenholm et al., 1984)
Naturally-contaminated wheat	6.8	0	Barrows, gilts	3 wk	↓FI	(Pollmann et al., 1985)
Naturally-contaminated wheat	3.7	0.4	Boars, gilts	23-53 kg	↓FI 25%	(Friend et al., 1986)
Naturally-contaminated corn	4.2	0.2	Boars, gilts	23-53 kg	↓FI 25%	(Friend et al., 1986)

[1] Adapted from (Diekman and Green, 1992).
[2] Unless otherwise stated, diets consisted of naturally contaminated field corn, or crystalline DON was added to achieve desired concentrations.
[3]ADG, average daily gain; FE, feed efficiency.

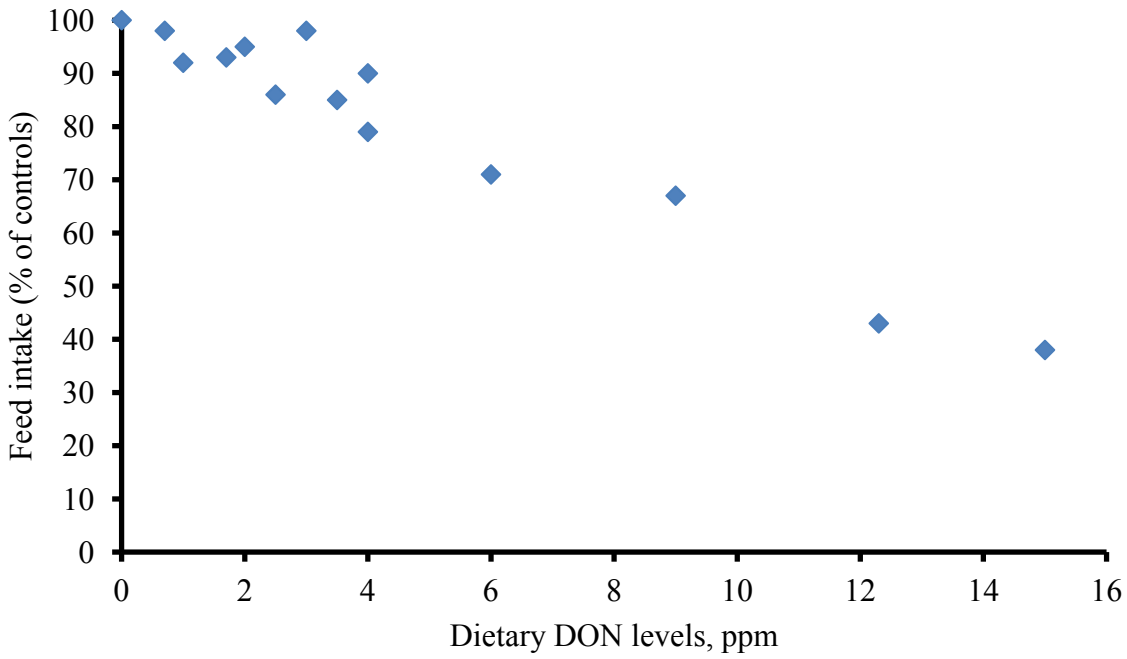


Figure 2.8. Effects of dietary deoxynivalenol (DON) levels on voluntary feed intake in pigs (D'Mello et al., 1999). Data selected from Bergsjo et al., 1993; Bergsjo et al., 1992; Friend et al., 1992; Prelusky et al., 1994; Rotter et al., 1995; Trenholm., 1994.

The effect of fumonisin on feed intake is summarized in Table 2.8. Several studies were conducted by using naturally contaminated feed, cultured material, or pure FB_1. Most of the studies presented fumonisin concentrations as FB_1. In the studies conducted, dietary concentrations of fumonisin varied from 0 to 200 ppm. However, with only one exception, only low (0 vs. 0.5-10 ppm) and high (0 vs. 100 or 200 ppm) concentrations were tested.

There is sufficient evidence that FB_1 concentrations of 175-200 ppm can have detrimental effects on pig performance (Colvin et al., 1993; Motelin et al., 1994). But the effects with lower concentrations are not consistent between studies, suggesting that in some studies there might be the appearance of some other mycotoxin in the diet. The effects of reduced growth rate at high dietary fumonisin concentrations are clearly related

to both feed intake (Colvin et al., 1993; Motelin et al., 1994) and an increase in feed:gain (Motelin et al., 1994). Consuming diets with pure FB_1 concentrations of 0, 0.11, 0.33, and 1 ppm, did not affect feed intake of barrows from 25 kg initial weight to 101 kg final weight (Rotter et al., 1997). However, the variation of feed intake increased when dietary FB_1 increased from 0 to 1 ppm. Another study found a linear decrease in feed intake in male pigs fed FB_1 up to 10 ppm, but not in females, though an apparent decrease was observed (Table 2.2.6)(Rotter et al., 1996b). In contrast, a different pattern was observed in another experiment where they examined dietary FB_1 concentrations of 10, 20 and 40 ppm on the performance of weaned pigs for 4 weeks. They reported no effects on feed consumption, though mild and severe pulmonary edema was found in pigs fed FB_1 diets (Kovacs et al., 2000). Curiously, in two studies an increase in feed intake was observed when pigs consumed low FB_1 concentrations at the initial stage compared to a toxin-free control diet (Prelusky et al., 1996; Rotter et al., 1996b).

Table 2.8. Summary of selected references on Fumonisin B_1 (FB_1) and feed intake (FI) in swine.[1]

FB_1 range (ppm)	No. of concentrations	Initial BW (Kg)	ADFI[2] (g/d)	R^2	Duration of study (weeks)	Reference
FB_1 from naturally contaminated material						
<1-136	6	6-13	-7.3	0.20	2	(Motelin et al., 1994)
FB_1 from cultured material						
0-100	2	17.7	-5.2		5	(Harvey et al., 1995)
0-200	2	13.2	-		3	(Colvin et al., 1993)
0-2.5[3]	2	±12	96.0		3.4	(Prelusky et al., 1996)
Purified FB_1						
0-10	4	16.3	-18.0	0.65	8	(Rotter et al., 1996b), males
0-10	4	14.4	-5.0	0.18	8	(Rotter et al., 1996b), females
0-1.0	4	25.6	9.8	0.09	11	(Rotter et al., 1997)

[1] Adapted from Dersjant-Li et al., 2003.
[2] Change per ppm increase in FB_1 concentration.
[3] Pigs in this group received 3mg ^{14}C-labelled FB1/kg feed from day 1 to 12, and 2mg ^{14}C-labelled FB1/kg feed from day 13 to 24.

Zearalenone effects on feed intake have not yet been well studied and few papers report feed intake changes when zearalenone is applied to the diet. A study conducted on boars fed diets containing 0, 3, 6, or 9 ppm of zearalenone from 32 days of age up to 145 or 312 days of age showed no significant differences between treatment in feed intake (Young and King, 1986). Another trial was conducted to test the effect of dietary protein concentration in diets containing either 0 or 50 ppm of zearalenone in 5 week old gilts, and no significant differences were found in terms of feed intake between treatments (Smith, 1980). In 1990, Young et al. conducted another experiment that supports the concept that zearalenone does not affect feed intake in pigs. In this study 48 parity 1 lactating sows were used to compare the effects of three dietary concentrations of

purified zearalenone (0, 5, and 10 ppm) with or without added dehydrated alfalfa. Feed intake in sows from day 7 of lactation until weaning at day 28 was 3.93, 3.80, and 3.69kg, for 0, 5, and 10 ppm, respectively.

In conclusion, feed containing deoxynivalenol concentrations of 1 ppm or higher results in significant reduction of feed intake from weaning to finisher pigs. More studies need to be conducted to determine the minimum concentration of fumonisin that can start affecting feed consumption. Nevertheless, results between studies are consistent when concentrations in feed are higher than 100 ppm, indicating that there is a reduction on feed intake. With regard to zearalenone, more investigation has to be done, but the few studies found do not show effects on feed intake.

2.2.4 Mycotoxins and nutrient digestibility

Some dose response studies have been conducted to determine the effects of deoxynivalenol on nutrient digestibility. Results has shown that feed with DON-concentrations of 1, 2 .3, and 4.6 ppm during the starter period (14 days) and concentrations of DON 0/0, 1.2/1.4, 2.3/3.7 ppm in the starter/grower diets (from 15 to 56 days) using naturally-contaminated wheat did not have a significant effect on nutrient digestibility (Danicke et al., 2004b). Another experiment testing DON-concentrations of 0.2 and 3.7 ppm with artificially-inoculated wheat observed the same result with no differences being found in nutrient digestibility in pigs with a live weight of 104 kg (Danicke et al., 2004a). A higher concentration of DON (18.53 ppm) and a non-contaminated feed were used for evaluating nutrient digestibility in pigs fed under ad libitum or restrictive feeding during 11 weeks with a live weight ranging between 26 to

100kg. A balance trial was conducted at the end of the experiment. A significant increase of metabolizable energy, digestibility of organic matter, crude protein, crude fat and N-retention by 4, 3, 6, 11 and 10% respectively was observed in the DON group of the restrictively fed pigs (Goyarts and Dänicke, 2005).

In 2007, Gbore and Egbunike, investigated the effect of fumonisin B_1 on nutrient digestibility in pigs of 8-9 weeks of age. Pigs were fed diets containing 0.2, 5.0, 10.0, and 15.0 ppm of FB_1 for 6 months, three physiological phases were determined, and a balance trial was conducted the last 7 days of each phase. In the first phase (weanling phase) there was a significant influence of the dietary FB_1 levels on the apparent digestibility of ether extract (EE). Animals on the control diet had higher apparent digestibility than those on the other three diets containing higher levels of FB_1, (67.91, 63.93, 62.83, 61.14% respectively). In phase two (peri-pubertal phase) the digestibility of the EE and crude protein (CP) were significantly ($P<0.05$) lower with increased dietary FB_1. Also, the apparent digestibility values observed during the last phase (pubertal phase) for animals on the control diet were generally higher (except for ash) than those on diets with FB_1.

Two studies have been conducted to determine the effect of the intake of zearalenone on nutrient digestibility. The first study showed the effect of feeding a concentration of 1 ppm of zearalenone for 36 days to pigs of 8.84 kg weaned at 21 days, and they did not observe any effect on nutrient digestibility (Jiang et al., 2012). On the contrary, another study showed effects of zearalenone stating that there is a significant diminution on dry matter, energy (85.9, 84.0, 83.4, 83.1% ,), and crude protein (85.6,

83.4, 81.8, and 81.2%,) digestibility in growing pigs, when different levels (0, 1, 2, and 3 ppm, respectively) of zearalenone were added to the diet (Chi and Yang, 2010).

There is a limited amount of clear and consistent information on the effects that different mycotoxins have on nutrient digestibility, and it is unclear for zearalenone and fumonisin what concentrations begin to affect nutrient digestibility. In terms of deoxynivalenol, there is also limited evidence about which concentrations affect pigs. Additional research with low levels of naturally-contaminated grains would be useful for swine.

CHAPTER 3. Effect of feeding a blend of naturally-contaminated corn on nutrient digestibility and feed preference in weanling pigs

3.1. Introduction

Mycotoxins are low molecular weight secondary metabolites produced by certain filamentous strains of fungi such as *Aspergillum*, *Penicillium*, and *Fusarium* which can invade crops and can grow during crop growth and grain storage if the appropriate environmental conditions (such as temperature and humidity) are present. Mycotoxins are estimated to affect 25% of the world crops each year (Lawlor and Lynch, 2005; Okoli et al., 2005) and every region of the world is susceptible to them. The economic losses due to the adverse effects on animal health and production have been recognized in different species such as swine, poultry, and cattle as a consequence of the consumption of high levels of mycotoxins in the diets (Smith et al., 1995). Susceptibility to mycotoxins varies depending on the species, physiological stage, genetic, and environmental factors, as well as the chemical structure of the mycotoxin. The biological effect can vary from carcinogenic, teratogenic, mutagenic, estrogenic, neurotoxic, or immunotoxic (Yiannikouris and Jouany, 2002a).

It is well documented that the appearance of different mycotoxins in feed can lead to a decrease of feed intake in pigs. Studies show that there are synergistic interactions between DON and fusaric acid leading to low feed intake and growth performance in growing pigs (Smith et al., 1997; Swamy et al., 2002). In 2010 a study mentioned that corn with DON is unpalatable to pigs, feed intake is reduced, and results in poor weight gain or even weight loss and an increase in digestive disorders (Gutzwiller, 2010). Also

inclusion of fumonisins and zearalenone induces a decrease in feed consumption (Jiang et al., 2011). Rats have been reported to discriminate between non-contaminated grains and grains contaminated with *Fusarium* sp., *F. roseum graminearum*, and *F. culmoru.* (Forsyth, 1974; Kotsonis et al., 1975; Roine et al., 1971). It was also found that pigs have a preference for non-contaminated corn rather than one containing trichothecenes (Vesonder et al., 1979). There is plenty of information on t he effect of different mycotoxins in growth performance and feed intake in pigs, but there are little data about pigs' ability to discriminate mycotoxin-contaminated feed. Also, the effect on nut rient digestibility in pigs is not yet well studied. In 2010, a study of Chi and Yang, showed the effects of zearalenone on dry matter, energy, and crude protein digestibility in growing pigs, stating that there is a significant diminution in these nutrient digestibilities when different levels (0, 1, 2, and 3 ppm) of zearalenone are added to the diet. Another study suggested that low concentrations of deoxynivalenol (1 to 4.6 ppm) do not affect nutrient digestibility in pigs, while concentrations greater than 18 ppm showed a significant increase of metabolizable energy, and crude protein (Dänicke et al., 2004a).

Therefore, the purpose of the current research was to evaluate the effect of feeding a naturally-contaminated corn produced in 2009 to a better quality corn produced in 2010 on diet preference and digestibility of dry matter, energy, and nitrogen in young pigs.

Two experiments were conducted to determine the effect of feeding diets with a 2009 and 2010 naturally-contaminated corn containing deoxynivalenol, zearalenone, 15-acetyl DON and fumonisin B_1 to weaning pigs. The 2009 corn contained more of the mycotoxins than the 2010 corn. For both experiments a total of three diets were mixed. Diets were blended to contain 100% 2010 naturally-contaminated corn (control), 50-50% blend of the 2009 naturally-contaminated corn and 2010 corn (Diet 2), and 100% 2009 naturally-contaminated corn (Diet 3). In Exp. 1, 24 crossbred pigs with an average body weight of 7.64 ± 0.70 kg were allotted to 4 replicates of 3 treatments with 2 pigs per pen, on the basis of gender, litter mate, and BW in a randomized complete block design for an experimental period of 20 d. Fecal and urine samples were collected daily and dry matter, energy, and nitrogen apparent digestibility were determined. Dry matter, energy, and nitrogen digestibility were not affected by either the 100% 2009 corn diet (Diet 3) or the 50% 2009 corn (Diet 2) compared to the 100% 2010 corn (Control). In Exp. 2, 30 crossbred pigs with an average body weight of 7.98 ± 1.15 kg were allotted to 3 replicates of 2 comparisons with 5 pigs per pen for 3 experimental periods of 1 week each. Comparisons consisted of: 1) Control vs Diet 3, and 2) Control vs Diet 2. Two feeders were located in each pen, each containing one of the two diets in order to make the mentioned comparisons; animal and feeder weights were recorded weekly to determine feed preference and growth performance. A preference for the feed containing 2010 corn feed was observed; when pigs were given the choice between feed containing the more highly contaminated 2009 corn vs feed containing the 2010 corn, they showed the ability to discriminate mycotoxin-contaminated feed (95.34 vs. 4.66%; $P< 0.01$) over the 3 week period. The discrimination was evident in each weekly period. Nutrient digestibility was

not affected by these mycotoxins in these levels, but a clear decrease in feed intake was observed in the pigs.

3.2 Materials and methods

The experiment was conducted under protocols approved by The University of Kentucky's Institutional Animal Care and Use Committee. Pigs were brought into the University of Kentucky nursery facility and placed in an environmentally-controlled room at approximately 3 weeks of age (weaning). The pigs were immediately placed on a complex nursery diet adequate in all nutrients.

3.2.1 Animals and dietary treatments

Experiment 1: This experiment (experiment ID: UK1103a) was carried out in February of 2011 and utilized a total of 24 crossbred pigs [12 barrows, 12 gilts; Yorkshire x Duroc; (Yorkshire x Landrance) x Duroc; (Yorkshire x Duroc) x Chester White; (Yorkshire x Landrance x Duroc) x Chester White], with an initial body weight (BW) of 7.64 ± 0.70 kg. The pigs were blocked by gender, BW, and breed of sire and randomly allotted to one of the three dietary treatments in a randomized complete block design. Pigs were fed in a nursery room for 5 days, and then moved to a room containing stainless steel metabolic pens (49 x 37cm), with each pen containing 2 pigs (either barrows or gilts) for 20 days (5 periods of 4 days each). Pigs were provided with *ad libitum* access to feed and water (nipple waterers) in the first 3 periods; in Periods 4 and 5 feed allowance was restricted and determined based on mean pen BW% (5% for Replicate 2, and 6% for Replicates 1, 3 and 4). Pigs were fed a complex nursery diet

based on NRC (1998) nutrient requirements for pigs with initial weight from 5 to 10 kg. Diets utilized 100% 2010 naturally-contaminated corn (control), 50-50% blend of the 2009 naturally-contaminated corn and 2010 corn (Diet 2), or 100% 2009 naturally-contaminated corn (Diet 3) (Table 3.2). The 2009 corn was more highly contaminated with mycotoxins as compared with the 2010 corn.

Experiment 2: This experiment (experiment ID: UK1103) was carried out in February of 2011 and utilized a total of 30 crossbred pigs [15 barrows, 15 gilts; Yorkshire x Duroc; (Yorkshire x Landrance) x Duroc; (Yorkshire x Duroc) x Chester White; (Yorkshire x Landrance x Duroc) x Chester White], with an initial body weight (BW%) 7.98 ± 1.15 kg. The pigs were allotted to one of the two dietary comparisons, Control vs Diet 3 (Comparison 1), and Control vs Diet 2 (Comparison 2) on the basis of BW in a randomized complete block design. Each comparison involved 3 pens, each one with 5 pigs (barrows and gilts combined). Pigs were fed in a nursery room for 5 days, and then allotted to each comparison. Pigs were provided with *ad libitum* access to feed and water for each of the three week periods. The diets were the same as in Exp 1 and pigs were fed a complex nursery diet based on NRC (1998) standards for pigs with initial weights from 5 to 10 kg. Diets determined to contain 100% 2010 naturally-contaminated corn (Control), 50-50% blend of the 2009 naturally-contaminated corn and 2010 corn (Diet 2), or 100% 2009 naturally-contaminated corn (Diet 3). Two comparisons were made: Control vs Diet 3 (Comparison 1), and Control vs Diet 2 (Comparison 2). The two diets were supplied in two different feeders in order to determine feed preference. Feeder

location was switched 3 times a week in order to eliminate the potential behavioral feeding pattern on pig preference.

Table 3.1. Composition of experimental diets for nursery pigs (%, as-fed basis)

	Diet		
Ingredients	1	2[1]	3[2]
Ground corn (2010, good quality)	57.13	28.56	-
Ground corn (2009, contaminated)	-	28.56	57.13
Soybean meal, 48% CP	19.35	19.35	19.35
Fish meal, menhaden	4.00	4.00	4.00
Animal plasma AP-920	2.50	2.50	2.50
Dried whey	12.50	12.50	12.50
Choice white grease	1.95	1.95	1.95
Dicalcium phosphate	0.90	0.90	0.90
Limestone	0.60	0.60	0.60
Salt	0.21	0.21	0.21
Trace mineral premix[3]	0.05	0.05	0.05
Vitamin premix[4]	0.08	0.08	0.08
Choline chloride 60%	0.05	0.05	0.05
Santoquin[5]	0.02	0.02	0.02
Mecadox-10[6]	0.25	0.25	0.25
L-Lysine.HCl	0.19	0.19	0.19
L-Threonine	0.09	0.09	0.09
DL-Methionine	0.14	0.14	0.14
Total:	100.00	100.00	100.00
Calculated nutrient composition			
ME, kcal/kg	3,394	3,394	3,394
Crude protein, %	20.24	20.24	20.24
Lysine, %	1.23	1.23	1.23
Calcium, %	0.80	0.80	0.80
Phosphorus, %	0.70	0.70	0.70
Available phosphorus, %	0.45	0.45	0.45
Analyzed nutrient composition			
Dry matter, %	88.77	88.10	86.75
Gross energy kcal/kg	4,030.29	3,990.06	3,942.89
Crude protein, %	21.01	20.71	21.23

[1] Diet 2 is a 50:50 blend of Diets 1 and 3, respectively.
[2] Mycotoxins natural-contaminated corn (see Table 3.2).
[3] Supplied per kg of diet: Zn, 150 mg as ZnO; Fe, 120 mg as $FeSO_4 \cdot H_2O$; Mn, 45 mg as MnO; Cu, 12 mg as $CuSO_4 \cdot 5H_2O$; I, 1.5 mg as CaI_2O_6; Se, 0.30 mg as $NaSeO_3$.
[4] Supplied per kg of diet: vitamin A, 6,600 IU; vitamin D_3, 880 IU; vitamin E, 44 IU; vitamin K (as menadione sodium bisulfate complex), 6.6 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B_{12}, 33 μg; folic acid, 1.3 mg; niacin, 44 mg; pantothenic acid, 22 mg; D-biotin, 0.22 mg.
[5] Provided 130 mg ethoxyquin per kilogram of diet.
[6] Mecadox-10, (Phibro Animal Health, Fairfield, NJ).Supplied 50 g of Carbadox/ton of diet.

Table 3.2. Mycotoxin concentration in 2009 and 2010 corn[1][2]

Mycotoxin	2009 corn, ppm	2010 corn, ppm	Diet 1 with 100% of 2010 corn, ppm	Diet 2 with 50% of 2009 and 50% 2010 corn, ppm	Diet 3 with 100% of 2009 corn, ppm	Critical levels for young growing pigs, ppm[3]
Aflatoxin B_1	<0.02	<0.02	-	-	-	<0.1
DON	5.6	0.5	0.29	1.73	3.19	<1
15-Acetyl DON	0.5	-	-	0.14	0.28	No reports
Fumonisin B_1	5.5	2.0	1.14	2.14	3.14	<10
Zearalenone	2.45	-	-	0.7	1.40	<1[4]

[1]Corn was analyzed by the Veterinary diagnostic laboratory of the North Dakota State university.
[2]Mycotoxin values are calculated from the corn inclusion rates.
[3]Values taken from the FDA, updated 08-30-2011. No FDA action, advisory or guidance levels established for zearalenone in US feed. The critical levels are concentration in finished feed.
[4]Taken from Pork Industry Handbook, 2005.

3.2.2 Housing conditions

Experiment 1: A total of 12 metabolism pens, with two pigs each, were used to conduct this balance trial. Pens were made of stainless steel and had plastic-coated expanded-metal flooring and plastic feeders. Metabolism pens also had a window in each side panel, near the feeder, to allow visual contact between pigs in adjacent pens. Underneath the floor of the pens a sliding aluminum screen was placed to allow separation of feces/urine, along with a stainless steel funneled-pan used to direct the urine into a 10 L plastic bucket. The interior space of the pens was set up at its maximum, so pigs were able to move around.

Experiment 2: A total of 6 pens, with 5 pigs each, were used to conduct this trial. Pigs were housed in elevated nursery pens with plastic coated, welded wire flooring (1.22 m x 1.22 m). Each was equipped with a nipple waterer and a single sided, three–hole plastic and metal feeder.

3.2.3 Adaptation and collection procedures

Experiment 1**:** Pigs were housed in a nursery room for a period of 5 days in order to get used to the complex nursery diet and standardize the GI tract. Then pigs were weighed, blocked by sex and weight, and randomly allotted to the metabolism pens and to one of the three diets.

The collection phase involved five periods of four days each. At the beginning and end of each period, pigs were weighed and 0.5% chromic oxide (Cr_2O_3) was added to the diet as a marker of the starting point of each collection period. Pigs were provided with *ad libitum* access to feed and water in the first 3 periods. In Periods 4 and 5 feed allowance was restricted and determined depending on their BW% (5% for Replicate 2, and 6% for Replicates 1, 3 and 4) and feed was provided in two meals per day. Rejected feed was dried in a forced-air oven at 55ºC, air-equilibrated, weighed, and discounted from the amount initially offered. All the feces produced during the period between excretion of the initial and final marker were collected daily and kept frozen in labeled plastic bags. Care was taken to include in the collected material all marked feces at the beginning of the collection period, as well as to exclude any marked feces at the end of the period. Urine was also collected on a daily basis in 10 L plastic buckets containing 50 mL of 3N HCl to limit microbial growth and reduce loss of ammonia. The total amount of daily urine was recorded and 100 mL subsamples were kept frozen in labeled, capped, plastic containers, while the rest of the urine was discarded.

Nutrient digestibility and retention (DM basis) by total collection were calculated using the formula:

Apparent digestibility, % = $\left[\frac{Nutrient\ Intake - Nutrient\ Excretion\ (Feces)}{Nutrient\ Intake}\right] \times 100$

Apparent retention, g/d = Nutrient intake, g/d – Total nutrient excretion (fecal + urinary; g/d)

Retention as a percent of intake, % = $\left[\frac{Nutrients\ retained}{Nutrient\ intake}\right] \times 100$

Retention as a percent of absorption, % = $\left[\frac{Nutrients\ retained}{Nutrient\ intake - Nutrient\ excretion\ (feces)}\right] \times 100$

Experiment 2: Pigs were housed in a nursery room for a period of 5 days in order to get used to the complex nursery diet and standardize the GI tract. Pigs were weighed and allotted to the nursery pens and to one of the two comparisons. The experiment length was three periods of 1 week each, at the beginning of each period animals and feeders were weighed. Also, the feeder location was switched 3 times a w eek in order to eliminate the potential behavioral feeding pattern on p igs' preference. The change of feeder location was video-recorded for an hour to observe animal behavior. The feeders were checked twice daily to remove waste in the feeder trough and to make sure the feed had not become blocked preventing normal flow. Water nipple heights were adjusted on an as-needed basis based on the growth of the pigs in each pen to ensure easy access.

3.2.4 Laboratory analysis

Experiment 1: Feed, feces, and urine were analyzed for dry matter, energy, and nitrogen content; the total contents of nutrients in feed, feces and urine, were calculated as the product of nutrient concentration by the total amount of material. Samples were analyzed in duplicate, and analysis was repeated when abnormal variation was observed.

All frozen feces were dried in a forced-air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) at 55°C for 7 days, then air-equilibrated, weighed, and ground through a 1 mm screen using a Wiley Laboratory Mill (Model 3, Arthur H. Thomas Co., Philadelphia, PA). After grinding, feces from each collection period were thoroughly mixed in a single bag for each pen. To obtain representative samples of urine for nutrient analysis, the daily samples were thawed at room temperature and proportionally pooled by weight for each pen according to the daily excretion recorded. Composited samples were kept frozen at all times until analyzed.

Samples were analyzed in duplicate, and analysis was repeated when a coefficient of variation higher than 5% was observed. Dry matter in feed and feces was assessed according to an adaptation of the AOAC (1995) method, involving overnight drying (105ºC) of the samples in a convection oven (Precision Scientific Co., Chicago, IL) and then calculating moisture contents as the difference between weighing. Apparent digestibility coefficients were calculated on a DM basis by using the equations detailed previously.

Gross energy content was assessed by bomb calorimetry, consisting of the ignition of samples in a pressurized-oxygen environment, and measuring the heat of combustion as the amount of energy transferred to a known mass of water contained in the

calorimeter, using benzoic acid as a standard (Model 1261 Isoperibol Bomb Calorimeter, Parr Instruments Company, Moline, IL).

To measure urine energy, samples were oven dried for 2 days at 55ºC into polyethylene flat bags prior to combustion. The known heat of combustion per gram of bag material was subtracted from the total heat observed to obtain the sample energy contents.

The nitrogen content of the diets, feces, and urine was determined using a gas combustion method, using glutamic acid as a st andard (AOAC, 1998; FP-2000, Leco Corp., St. Joseph, MI).

3.2.5 Statistical analysis

Experiment 1: The experimental data was analyzed using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Each metabolism pen was considered as an experimental unit for growth performance and digestibility measures. The statistical model included terms for diet, sex (replicate), and diet x sex (replicate). The linear and quadratic effects of diet were calculated. The alpha level used for determination of statistical significance was 0.05.

Experiment 2: The data was analyzed by unpaired T-tests using the GraphPad Prism program (GraphPad Software, Inc., San Diego, CA). The experimental unit was the pen. The statistical model included treatment and differences were considered significant at $\alpha = 0.05$.

3.3 Results

3.3.1 Experiment 1

In Period 1 as shown in Table 3.3, the average daily gain and feed intake were affected by the different diets ($P = 0.01$). The feed intake in pigs consuming the 2010 corn diet was greater (754.89 g/d) than the 50-50% blend with 2010 and 2009 corn, and the all 2009 corn diets (618.52 and 449.63 g/d, respectively). Average daily gain showed the same pattern as feed intake for the different diets (248.06, 186.40, and 62.37 g/d).

Apparent dry matter digestibility was not affected ($P > 0.10$), although the 2010 corn diet had the greatest numerical digestibility (90.45%). Apparent energy digestibility was also not affected ($P > 0.10$) despite an apparent stepwise reduction from the 2010 corn diet (90.09%) to the 50-50% blend and the all 2009 corn diets (89.20% and 88.89%, respectively).

Metabolizable energy percentage from digestible energy was linearly reduced ($P > 0.057$) with the 2010 corn diet having greater digestibility (97.56%) than the 50-50% blend with 2010 and 2009 corn, and the all 2009 corn diets (96.78% and 96.28%, respectively).

Apparent nitrogen digestibility was not affected by the diets (87.76, 87.27 and 87.84%; $P > 0.10$). The percentage of nitrogen retained from feed intake and absorbed was not affected by the diets ($P > 0.10$), but both showed a stepwise decrease from the 2010 corn diet than the 50-50% blend with 2010 and 2009 corn, and the all 2009 corn diets, respectively.

Table 3.3. Effect of naturally-contaminated corn on nutrient digestibility measures during Period 1[1].

Item	Diets			PSEM[2]	*P*-values		
	Control	2	3		Diet	Diet*Sex	Linear
Performance							
Initial weight, kg	7.65	7.64	7.64	0.04	0.946	0.094	0.803
Final weight, kg	8.65	8.38	7.89	0.12	0.025	0.212	0.011
ADG, g/d	248.06	186.40	62.37	22.52	0.010	0.318	0.004
Feed intake							
ADF, g/d	754.89	618.52	449.63	36.75	0.011	0.881	0.004
DMI, g/d	670.12	544.92	390.04	32.12	0.009	0.881	0.725
Wet fecal mass, g/d	131.88	119.74	75.80	12.70	0.073	0.659	0.036
Fecal DM, %[3]	49.63	48.19	51.90	1.44	0.294	0.104	0.328
Urine weight, g/d	1514.79	2005.82	1599.52	417.72	0.697	0.419	0.893
Urine DM, %	1.83	1.16	1.80	0.45	0.542	0.240	0.965
Urine DM, g/d	22.34	21.54	16.40	1.34	0.066	0.017	0.035
Apparent DM digestibility, %	90.45	89.61	89.81	1.33	0.900	0.997	0.752
Energy							
Intake, kcal/d	3042.40	2467.93	1719.57	163.74	0.012	0.982	0.005
Fecal energy, kcal/d	303.05	265.64	184.50	27.16	0.082	0.977	0.037
Apparent digestibility, kcal/d	2739.35	2202.29	1535.07	168.13	0.018	0.988	0.007
Apparent digestibility, %	90.09	89.20	88.89	1.68	0.876	0.984	0.641
DE, kcal/d	3630.96	3559.13	3394.38	130.04	0.486	0.750	0.268
Urine energy, kcal/d	66.72	71.10	54.94	3.37	0.060	0.035	0.069
ME, kcal/d	3542.36	3444.58	3270.00	135.08	0.432	0.793	0.227
Retained energy, kcal/d	2672.64	2131.19	1480.13	166.90	0.018	0.977	0.007
ME from DE, %	97.56	96.78	96.28	0.34	0.129	0.404	0.057
Nitrogen							
Intake, g/d	25.37	20.91	15.29	1.25	0.012	0.882	0.005
Fecal nitrogen, g/d	3.13	2.66	1.83	0.30	0.091	0.929	0.040
Apparent digestibility, %	87.76	87.27	87.84	1.68	0.967	0.978	0.975
Urine nitrogen, g/d	3.29	2.75	2.39	0.27	0.180	0.448	0.082
Retained nitrogen, g/d	18.96	15.50	11.06	1.38	0.038	0.925	0.016
Retained, % of intake	74.81	73.99	72.32	3.26	0.864	0.853	0.617
Retained, % of absorbed	85.23	84.72	82.27	2.44	0.682	0.762	0.439

[1] Each mean represent the average of 4 pens/treatment with 2 pigs/pen. Pigs were fed a complex nursery diet made with 2010 corn (Control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). The experimental period length was 4 days and feed was provided *ad libitum.*

[2] PSEM- Pooled standard error of the mean.

[3] Quadratic effect ($P < 0.10$).

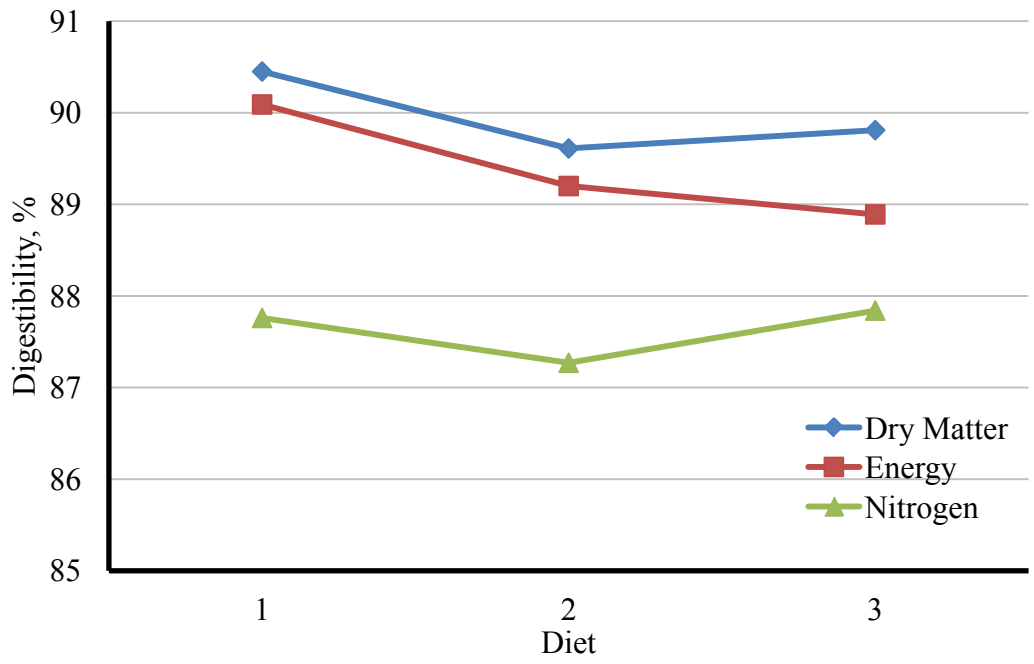


Figure 3.1. Effect of naturally-contaminated corn on DM, energy, and nitrogen apparent digestibility during Period 1. Each mean represents the average of 4 pens/treatment with 2 pigs/pen. The experimental period length was 4 days and feed was provided ad libitum. No diet effects were observed ($P > 0.10$).

In Period 2, as shown in Table 3.4, the average daily gain and feed intake was affected by the different diets ($P < 0.01$). The feed intake in pigs consuming the all 2010 corn diet was greater (820.49 g/d) than the 50-50% blend with 2010 and 2009 corn, and the all 2009 corn diets (719.68 and 575.76 g/d, respectively). Average daily gain showed the same pattern as feed intake for the different diets (463.52, 428.79, and 284.20 g/d).

Apparent dry matter digestibility was not affected ($P > 0.10$) but the pattern is reversed from Period 1 with the 2010 corn diet having lower digestibility (88.67%) than the 50-50% blend with 2010 and 2009 corn and the all 2009 corn diets (88.88% and 89.92%, respectively).

Apparent energy digestibility was not affected ($P > 0.10$) despite an apparent stepwise increase from the 2010 corn diet (88.11%) to the 50-50% blend and the all 2009 corn diets (88.33% and 88.72%, respectively).

Metabolizable energy percentage from digestible energy was not affected ($P > 0.10$); the 2010 corn diet and the 50-50% blend with 2010 and 2009 corn had similar percentages (96.72% and 96.95%, respectively) comparing to the diet with all 2009 corn that was lower in number (94.65%).

Apparent nitrogen digestibility was not affected by the diets (85.73, 87.00 and 87.53%; $P > 0.10$), despite an apparent stepwise increase from the 2010 corn diet to the 50-50% blend with 2010 and 2009 corn and the all 2009 corn diets respectively.

Table 3.5 presents the interactions that were found between diet by sex ($P < 0.05$); barrows showed greater daily feed intake than gilts in the all 2010 corn diet and the 50-50% blend with 2010 and 2009 corn diet (barrows: 910.37, gilts: 730.62g; barrows: 840.78, gilts: 598.58g, respectively); in the all 2009 corn diet the opposite occurs, but the feed intake was slightly higher for gilts (gilts: 594.58g, barrows: 556.95g). The rest of the variables shown in Table 3.5 show the same type of response, since all of these measures are related to the feed intake.

Table 3.4. Effect of naturally-contaminated corn on nutrient digestibility measures during Period 2.[1]

Item	Diets			PSEM[2]	*P*-values		
	Control	2	3		Diet	Diet*Sex	Linear
Performance							
Initial weight, kg	8.65	8.38	7.89	0.12	0.025	0.212	0.011
Final weight, kg	10.50	10.10	9.03	0.16	0.007	0.443	0.003
ADG, g/d	463.52	428.79	284.20	24.59	0.014	0.628	0.007
Feed intake							
ADF, g/d	820.49	719.68	575.76	21.26	0.003	0.021	0.001
DMI, g/d	728.36	634.05	499.46	18.65	0.003	0.020	0.001
Wet fecal mass, g/d	171.52	157.19	111.75	16.13	0.121	0.292	0.059
Fecal DM, %[3]	47.69	44.25	48.12	0.83	0.054	0.023	0.730
Urine weight, g/d	1821.15	2257.78	1748.08	256.33	0.402	0.967	0.850
Urine DM, %	1.89	1.06	2.35	0.36	0.141	0.277	0.414
Urine DM, g/d	30.36	21.36	38.12	7.76	0.398	0.509	0.518
Apparent DM digestibility, %	88.67	88.88	89.21	1.07	0.938	0.741	0.739
Energy							
Intake, kcal/d	3306.82	2871.56	2270.16	84.34	0.003	0.020	0.001
Fecal energy, kcal/d	384.83	326.87	253.77	28.36	0.074	0.305	0.031
Apparent digestibility, kcal/d	2921.99	2544.69	2016.40	87.79	0.005	0.033	0.002
Apparent digestibility, %	88.11	88.33	88.72	1.07	0.921	0.737	0.707
DE, kcal/d	3551.08	3524.52	3498.07	42.45	0.700	0.733	0.427
Urine energy, kcal/d	95.21	75.59	111.19	17.08	0.419	0.772	0.545
ME, kcal/d	3434.80	3417.08	3310.63	62.60	0.403	0.804	0.233
Retained energy, kcal/d	2826.78	2469.11	1905.21	97.24	0.007	0.042	0.003
ME from DE, %	96.72	96.95	94.65	0.80	0.197	0.520	0.141
Nitrogen							
Intake, g/d	27.57	24.33	19.58	0.72	0.004	0.021	0.001
Fecal nitrogen, g/d	3.85	3.09	2.42	0.34	0.098	0.439	0.042
Apparent digestibility, %	85.73	87.00	87.53	1.38	0.666	0.809	0.408
Urine nitrogen, g/d	3.84	3.10	5.05	1.19	0.556	0.502	0.512
Retained nitrogen, g/d	19.89	18.15	12.11	1.66	0.062	0.120	0.030
Retained, % of intake	71.75	74.17	62.60	6.27	0.461	0.437	0.361
Retained, % of absorbed	83.68	85.23	71.72	6.20	0.341	0.348	0.244

[1] Each mean represent the average of 4 pens/treatment with 2 pigs/pen. Pigs were fed a complex nursery diet made with 2010 corn (Control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). The experimental period length was 4 days and feed was provided *ad libitum.*

[2] PSEM- Pooled standard error of the mean.

[3] Quadratic effect ($P < 0.05$).

Table 3.5. Effect of naturally-contaminated corn and sex in digestibility measures during Period 2[1].

	Control		Diet 2		Diet 3			*P*-value
								Diet *
	Gilts	Barrows	Gilts	Barrows	Gilts	Barrows	PSEM[2]	Sex
ADF, g/d	730.62	910.37	598.58	840.78	594.58	556.95	30.06	0.021
DMI, g/d	648.58	808.14	527.36	740.74	515.78	483.13	26.37	0.020
Fecal DM, %	45.97	49.41	46.08	42.43	51.89	44.35	1.17	0.023
Energy intake, kcal/d	2944.60	3669.04	2388.37	3354.76	2344.35	2195.97	119.28	0.020
Apparent energy digestibility, kcal/d	2547.21	3296.76	2098.52	2990.87	2057.26	1975.54	124.15	0.033
Retained energy, kcal/d	2457.06	3196.50	2026.78	2911.44	1966.02	1844.40	137.52	0.042
Retained nitrogen, g/d	24.55	30.59	20.24	28.42	20.22	18.94	1.02	0.021

[1]Each mean represent 4 pens with 2 pigs/pen, diets were a complex nursery diet made with 2010 corn (control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). Experimental period length was 4 days. Fed *ad libitum*

[2]PSEM- Pooled standard error of the mean

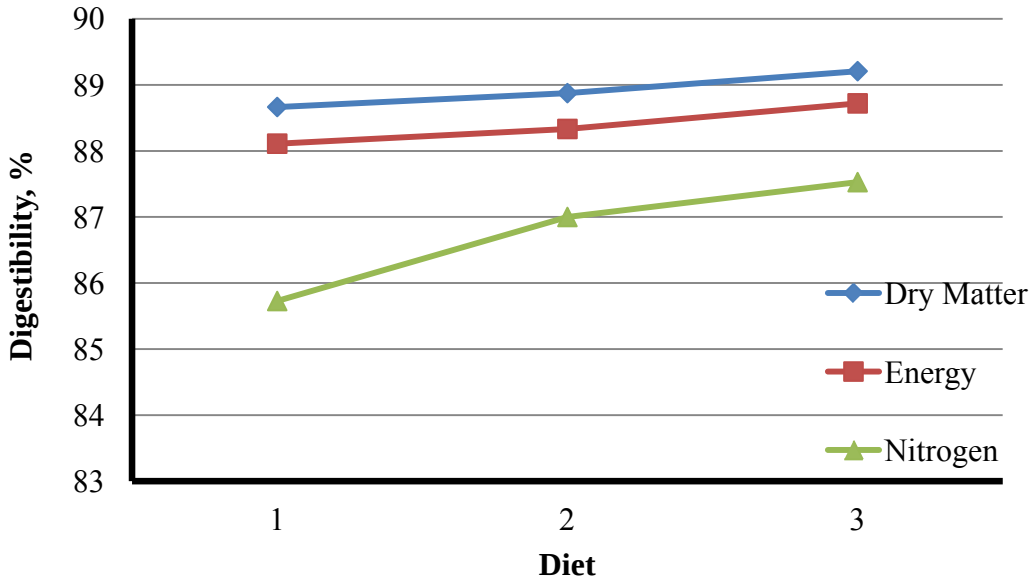


Figure 3.2. Period 2: Effect of naturally-contaminated corn on DM, energy, and nitrogen apparent digestibility. Each mean represents 4 pens with 2 pigs/pen. The experimental period length was 4 days and feed was provided ad libitum. No diet effects were observed ($P > 0.10$).

In Period 3, as shown in Table 3.6, feed intake was linearly affected ($P = 0.025$). The feed intake in pigs consuming the 2010 corn was greater (1696.75 g/d) than the 50-50% blend with 2010 and 2009 corn, and the all 2009 corn (1553.75 and 1334.51 g/d, respectively). Average daily gain showed the same pattern as feed intake for the different diets, but no difference was found ($P > 0.10$; 643.53, 574.78, and 521.63 g/d, respectively).

Apparent dry matter digestibility was not affected ($P > 0.10$), although the 2010 corn diet had a greater numerical digestibility (90.30%) than the 50-50% blend and the all 2009 corn diets (89.27% and 89.45%, respectively). However, data in Table 3.7 shows that differences were found in the interaction between diet by sex, ($P < 0.05$). Barrows showed greater apparent dry matter digestibility than gilts in the control diet and the all

2009 corn diet (barrows: 90.60, gilts: 90.00%; barrows: 91.33, gilts: 87.58%, respectively). Conversely, the 50-50% blend corn diet showed the opposite pattern, with gilts having a greater dry matter digestibility (barrows: 88.85, gilts: 89.69%).

Apparent energy digestibility was not affected ($P > 0.10$) and no patterns were observed, even though the 2010 corn diet showed a grater digestibility (90.12%) than the 50-50% blend and the all 2009 corn diet (89.17% and 89.46%, respectively). Table 3.7 shows significant differences in the interaction between diet by sex, ($P < 0.05$); barrows showed greater apparent energy digestibility than gilts in the control diet and the all 2009 corn diet (barrows: 90.45, gilts: 89.79%; barrows: 91.30, gilts: 87.62%, respectively). The 50-50% blend diet showed an opposite pattern: gilts showed greater apparent dry matter digestibility than barrows (barrows: 88.67, gilts: 89.68%, respectively).

Metabolizable energy percentage from digestible energy was not affected by diet ($P > 0.10$), and all diets were similar in percentage (97.85, 97.55 and 97.80% for control, 50-50% blend, and all 2009 corn diet, respectively). Apparent nitrogen digestibility was not affected by the diets (88.25, 87.47 and 88.14%; $P > 0.10$), although the 2010 corn had the greatest numerical digestibility. Nevertheless, Table 3.7 shows that differences were found in the interaction between diet by sex, ($P < 0.05$). Barrows showed a greater apparent nitrogen digestibility compared to gilts in the all 2010 corn diet and the all 2009 corn diet (barrows: 88.80, gilts: 87.69%; barrows: 90.55, gilts: 85.74%, respectively). The 50-50 % blend diet showed a different pattern, gilts showed slightly greater apparent nitrogen digestibility than barrows (gilts: 87.55, barrows: 87.40%, respectevely).

Table 3.6. Effect of naturally-contaminated corn on nutrient digestibility measures during Period 3.[1]

Item	Diets			PSEM[2]	*P*-values		
	Control	2	3		Diet	Diet*Sex	Linear
Performance							
Initial weight, kg	10.50	10.10	9.03	0.16	0.007	0.443	0.003
Final weight, kg	13.08	12.40	11.11	0.24	0.011	0.407	0.004
ADG, g/d	643.53	574.79	521.63	62.14	0.454	0.380	0.238
Feed intake							
ADF, g/d	1696.75	1553.75	1334.51	73.05	0.059	0.613	0.025
DMI, g/d	1506.22	1368.87	1157.65	64.22	0.045	0.610	0.019
Wet fecal mass, g/d	289.23	322.41	250.21	18.63	0.121	0.176	0.213
Fecal DM, %[3]	50.84	45.19	49.26	1.75	0.175	0.389	0.559
Urine weight, g/d[4]	2512.43	3404.01	2203.90	137.91	0.008	0.025	0.188
Urine DM, %	2.01	1.19	2.47	0.24	0.050	0.026	0.257
Urine DM, g/d	46.25	40.75	36.49	3.12	0.201	0.057	0.092
Apparent DM digestibility, %	90.30	89.27	89.45	0.31	0.148	0.015	0.123
Energy							
Intake, kcal/d	6838.38	6199.54	5261.83	291.02	0.045	0.609	0.019
Fecal energy, kcal/d	674.64	664.05	548.79	36.44	0.125	0.256	0.071
Apparent digestibility, kcal/d	6163.74	5535.49	4713.04	260.01	0.041	0.498	0.017
Apparent digestibility, %	90.12	89.17	89.46	0.33	0.237	0.018	0.234
DE, kcal/d	3631.88	3558.07	3526.98	13.35	0.012	0.019	0.005
Urine energy, kcal/d	135.06	137.59	104.69	11.48	0.194	0.277	0.135
ME, kcal/d	3553.49	3470.71	3449.26	12.10	0.008	0.014	0.004
Retained energy, kcal/d	6028.68	5397.90	4608.34	253.36	0.041	0.512	0.017
ME from DE, %	97.85	97.55	97.80	0.16	0.423	0.368	0.841
Nitrogen							
Intake, g/d	57.01	52.52	45.37	2.47	0.069	0.616	0.029
Fecal nitrogen, g/d	6.68	6.51	5.30	0.39	0.129	0.333	0.070
Apparent digestibility, %	88.25	87.47	88.14	0.38	0.384	0.022	0.854
Urine nitrogen, g/d	5.34	4.58	4.17	0.31	0.134	0.081	0.061
Retained nitrogen, g/d	44.99	41.43	35.90	2.12	0.091	0.728	0.039
Retained, % of intake	78.96	78.80	78.99	0.53	0.967	0.166	0.965
Retained, % of absorbed	89.49	90.10	89.66	0.79	0.858	0.264	0.891

[1]Each mean represent 4 pens with 2 pigs/pen, diets were a complex nursery diet made with 2010 corn (control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). Experimental period length was 4 days. Fed *ad libitum*

[2]PSEM- Pooled standard error of the mean

[3]Quadratic effect ($P < 0.10$).

[4]Quadratic effect ($P < 0.01$).

Table 3.7. Effect of naturally-contaminated corn and sex in digestibility measures[1].

	Control		Diet 2		Diet 3			*P*-value
	Gilts	Barrows	Gilts	Barrows	Gilts	Barrows	PSEM[2]	Diet* Sex
Urine weight, g/d	2938.98	2085.88	3560.53	3247.49	3242.62	1165.19	195.04	0.025
Apparent DM digestibility, %	90.00	90.60	89.69	88.85	87.58	91.33	0.44	0.015
Apparent energy digestibility, kcal/k	3618.63	3645.14	3578.32	3537.83	3454.46	3599.51	18.88	0.019
Apparent energy digestibility, %	89.79	90.45	89.68	88.67	87.62	91.30	0.47	0.018
ME, kcal/kg	3557.90	3549.09	3495.87	3445.55	3385.06	3513.46	17.11	0.014
Apparent nitrogen digestibility, %	87.69	88.80	87.55	87.40	85.74	90.55	0.54	0.022

[1]Each mean represent 4 pens with 2 pigs/pen, were a complex nursery diet made with 2010 corn (control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). Experimental period length was 4 days. Fed *ad libitum*

[2]PSEM- Pooled standard error of the mean

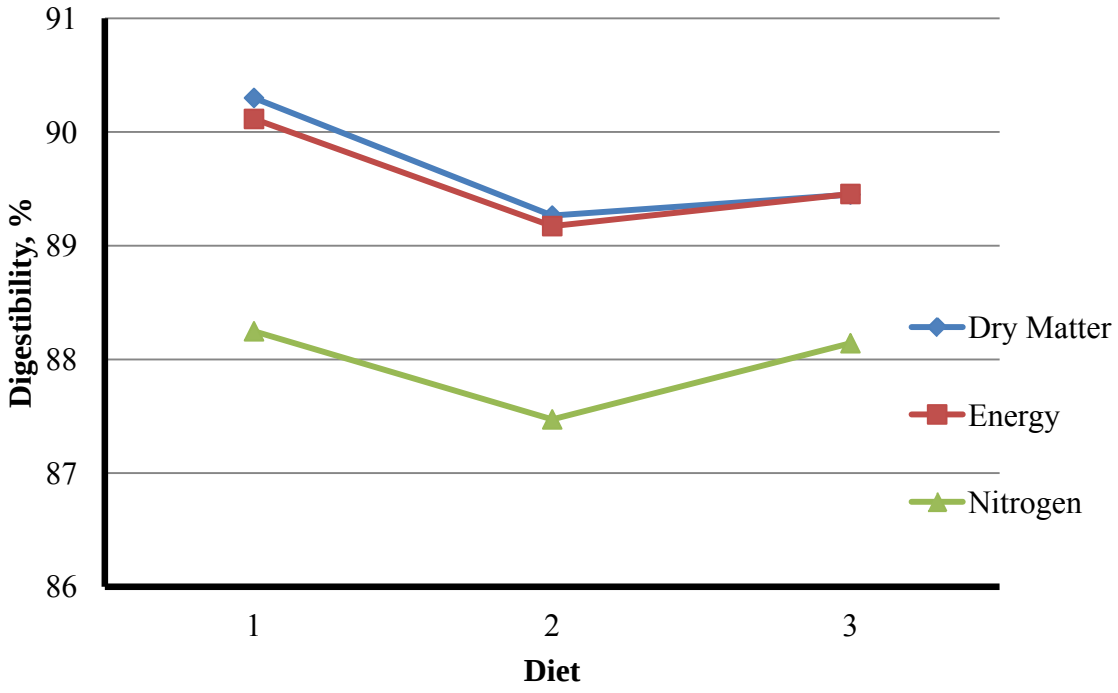


Figure 3.3. Period 3: Effect of naturally-contaminated corn on DM, energy, and nitrogen apparent digestibility. Each mean represent 4 pens with 2 pigs/pen. Experimental period length was 4 days and feed was provided ad libitum. No diet effects were observed (P > 0.10).

The results for Period 4 are shown in Table 3.8. The method of feeding was changed from *ad libitum* provision to scale feeding based on pig BW. Average daily gain was not affected by the different diets ($P > 0.10$; 514.54, 440.13, and 444.52g for the Control, 50-50% blend corn and the all 2009 corn respectively). Feed intake in pigs consuming the 2010 corn was greater (1380.84 g/d) than the 50-50% blend corn and the all 2009 corn (1307.74 and 1021.85 g/d, respectively), and a linear response was observed ($P < 0.05$).

Apparent dry matter and energy digestibility was not affected by the diets ($P > 0.10$), although the 2010 corn diet had the greatest numerical digestibilities in both cases (88.91%, and 88.67%, respectively). Also, metabolizable energy percentage from digestible energy was not affected ($P > 0.10$), despite an apparent stepwise reduction from the 2010 corn diet (96.55, 97.31 and 96.84%, respectively).

Apparent nitrogen digestibility was not affected by the diets (86.53, 84.07 and 85.98%; $P > 0.10$), although the all 2010 corn diet had the greatest numerical digestibility comparing to the other two diets.

The percentage of nitrogen retained from feed intake and absorbed was not affected by the diets ($P > 0.10$), despite an apparent stepwise reduction from the all 2010 corn diet to the 50-50% blend and the all 2009 corn diet (retained from intake: 74.85, 71.70 and 69.92%; retained from absorbed: 86.52, 85.35 and 81.30%, respectively).

Table 3.8. Effect of naturally-contaminated corn on nutrient digestibility measures during Period 4.[1]

Item	Diets			PSEM[2]	*P*-values		
	Control	2	3		Diet	Diet*Sex	Linear
Performance							
Initial weight, kg	13.08	12.40	11.11	0.24	0.011	0.407	0.004
Final weight, kg	15.13	14.16	12.89	0.25	0.008	0.288	0.003
ADG, g/d	514.54	440.13	444.52	61.69	0.662	0.780	0.467
Feed intake							
ADF, g/d	1380.84	1307.74	1021.85	78.41	0.065	0.603	0.032
DMI, g/d	1225.79	1152.14	886.42	68.89	0.053	0.608	0.025
Wet fecal mass, g/d	260.59	351.67	251.80	43.73	0.309	0.847	0.894
Fecal DM, %[3]	53.88	45.78	45.35	3.65	0.286	0.919	0.173
Urine weight, g/d	3278.12	3588.23	2817.88	289.65	0.278	0.122	0.324
Urine DM, %	1.41	1.14	2.06	0.41	0.353	0.300	0.318
Urine DM, g/d	41.83	39.57	38.31	3.30	0.761	0.920	0.492
Apparent DM digestibility, %	88.91	86.37	87.30	0.61	0.097	0.976	0.137
Energy							
Intake, kcal/d	5565.17	5217.98	4029.04	312.59	0.054	0.607	0.026
Fecal energy, kcal/d	629.44	727.86	519.41	49.55	0.097	0.791	0.192
Apparent digestibility, kcal/d	4935.73	4490.11	3509.63	277.06	0.050	0.602	0.022
Apparent digestibility, %	88.67	86.13	87.14	0.61	0.099	0.997	0.152
DE, kcal/d	3573.62	3436.76	3435.70	24.35	0.025	0.997	0.016
Urine energy, kcal/d	121.71	119.75	108.79	5.95	0.353	0.355	0.200
ME, kcal/d	3485.89	3344.10	3327.21	28.67	0.032	0.977	0.017
Retained energy, kcal/d	4814.03	4370.37	3400.84	278.83	0.053	0.613	0.023
ME from DE, %	97.55	97.31	96.84	0.31	0.354	0.802	0.180
Nitrogen							
Intake, g/d	46.40	44.21	34.74	2.65	0.072	0.601	0.036
Fecal nitrogen, g/d	6.24	7.09	4.86	0.55	0.106	0.902	0.152
Apparent digestibility, %	86.53	84.07	85.98	0.83	0.205	0.920	0.664
Urine nitrogen, g/d	5.43	5.37	5.42	0.35	0.994	0.344	0.985
Retained nitrogen, g/d	34.73	31.74	24.47	2.52	0.098	0.740	0.045
Retained, % of intake	74.85	71.70	69.92	1.81	0.264	0.980	0.127
Retained, % of absorbed	86.52	85.35	81.30	1.95	0.253	0.989	0.131

[1]Each mean represent 4 pens with 2 pigs/pen. Diets were a complex nursery diet made with 2010 corn (control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). Experimental period length was 4 days. Fed by BW% (5% for rep 2, and 6% for replicates 1, 3 and 4).

[2]PSEM- Pooled standard error of the mean.

[3]Quadratic effect ($P < 0.10$).

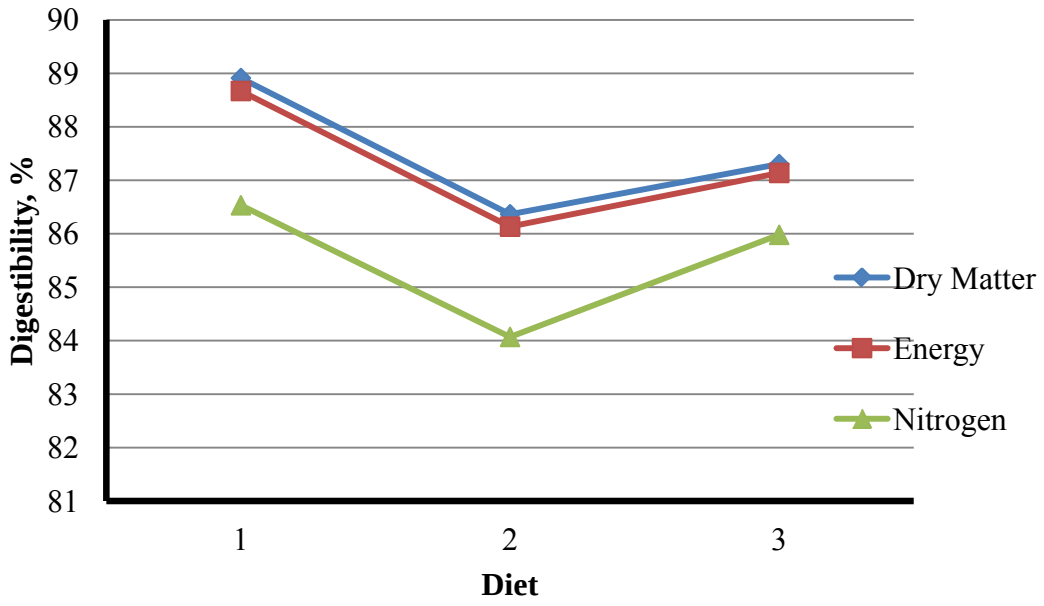


Figure 3.4. Period 4: Effect of naturally-contaminated corn in DM, energy, and nitrogen apparent digestibility. Each mean represent 4 pens with 2 pigs/pen. Experimental period length was 4 days and feed was provided depending on BW% (5% for Replicate 2, and 6% for Replicates 1, 3 and 4). No diet effects were observed (P > 0.10).

In Period 5, as shown in Table 3.9, average daily gain was not affected by the different diets (P > 0.10; 579.04, 633.61 and 461.25g, for the Control, 50-50% corn and the all 2009 corn, respectively). Feed intake in pigs consuming the 2010 corn was greater (1471.66 g/d) than in pigs consuming the 50-50% blend and the all 2009 corn diet, respectively (1402.18 and 1162.12 g/d) but no difference was found (P > 0.10).

Dry matter, energy and nitrogen apparent digestibility was not affected (P > 0.10) despite an apparent stepwise reduction from the 2010 corn (89.80, 89.55 and, 88.13%, respectively), to the 50-50% blend and the all 2009 corn.

Metabolizable energy percentage from digestible energy was not affected (P > 0.10), and no pattern was observed (96.66, 96.67 and 96.57% respectively). Also, the percentage of nitrogen retained from feed intake and absorbed, was not affected by diets

($P > 0.10$). However the 50-50% blend corn diet was numerically higher (retained from intake: 75.04%; retained from absorbed: 85.29%) than the all 2009 corn diet, and the all 2010 corn diet respectively.

Table 3.9. Effect of naturally-contaminated corn on nutrient digestibility measures during Period 5.[1]

Item	Diets			PSEM[2]	*P*-values		
	Control	2	3		Diet	Diet*Sex	Linear
Performance							
Initial weight, kg	15.13	14.16	12.89	0.25	0.008	0.288	0.003
Final weight, kg	17.45	16.69	14.74	0.30	0.008	0.228	0.003
ADG, g/d	579.04	633.61	461.25	45.51	0.121	0.229	0.141
Feed intake							
ADF, g/d	1471.66	1402.18	1162.12	91.02	0.149	0.720	0.074
DMI, g/d	1306.41	1235.34	1008.10	80.75	0.122	0.727	0.059
Wet fecal mass, g/d	230.89	256.55	237.81	38.08	0.888	0.770	0.904
Fecal DM, %[3]	57.55	51.57	50.06	2.67	0.227	0.426	0.119
Urine weight, g/d	4244.11	4550.46	3567.70	725.95	0.651	0.582	0.546
Urine DM, %	1.52	1.07	2.08	0.61	0.558	0.440	0.554
Urine DM, g/d	54.32	47.66	43.12	2.06	0.044	0.095	0.018
Apparent DM digestibility, %	89.80	89.02	88.26	1.30	0.723	0.511	0.449
Energy							
Intake, kcal/d	5931.21	5594.77	4582.10	366.23	0.124	0.727	0.060
Fecal energy, kcal/d	593.24	606.11	549.25	63.93	0.814	0.548	0.652
Apparent digestibility, kcal/d	5337.97	4988.67	4032.85	385.76	0.156	0.692	0.075
Apparent digestibility, %	89.55	88.85	88.10	1.35	0.762	0.491	0.488
DE, kcal/d	3608.90	3544.97	3473.48	54.10	0.314	0.494	0.152
Urine energy, kcal/d	173.82	160.69	136.89	10.71	0.157	0.654	0.071
ME, kcal/d	3488.58	3427.10	3354.50	54.34	0.322	0.523	0.156
Retained energy, kcal/d	5164.15	4827.98	3895.96	380.77	0.162	0.698	0.078
ME from DE, %	96.66	96.67	96.57	0.26	0.965	0.928	0.845
Nitrogen							
Intake, g/d	49.45	47.39	39.51	3.06	0.164	0.716	0.083
Fecal nitrogen, g/d	5.61	5.59	5.11	0.66	0.838	0.620	0.619
Apparent digestibility, %	88.13	87.87	87.09	1.73	0.909	0.562	0.692
Urine nitrogen, g/d	9.16	5.67	5.54	1.52	0.272	0.667	0.166
Retained nitrogen, g/d	34.68	36.13	28.86	3.47	0.383	0.486	0.301
Retained, % of intake	69.86	75.04	72.83	3.41	0.601	0.375	0.572
Retained, % of absorbed	79.32	85.29	83.60	3.85	0.575	0.547	0.475

[1]Each mean represents 4 pens with 2 pigs/pen. Diets were a complex nursery diet made with 2010 corn (control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). Experimental period length was 4 days. Fed by BW% (5% for rep 2, and 6% for replicates 1, 3 and 4).

[2]PSEM- Pooled standard error of the mean.

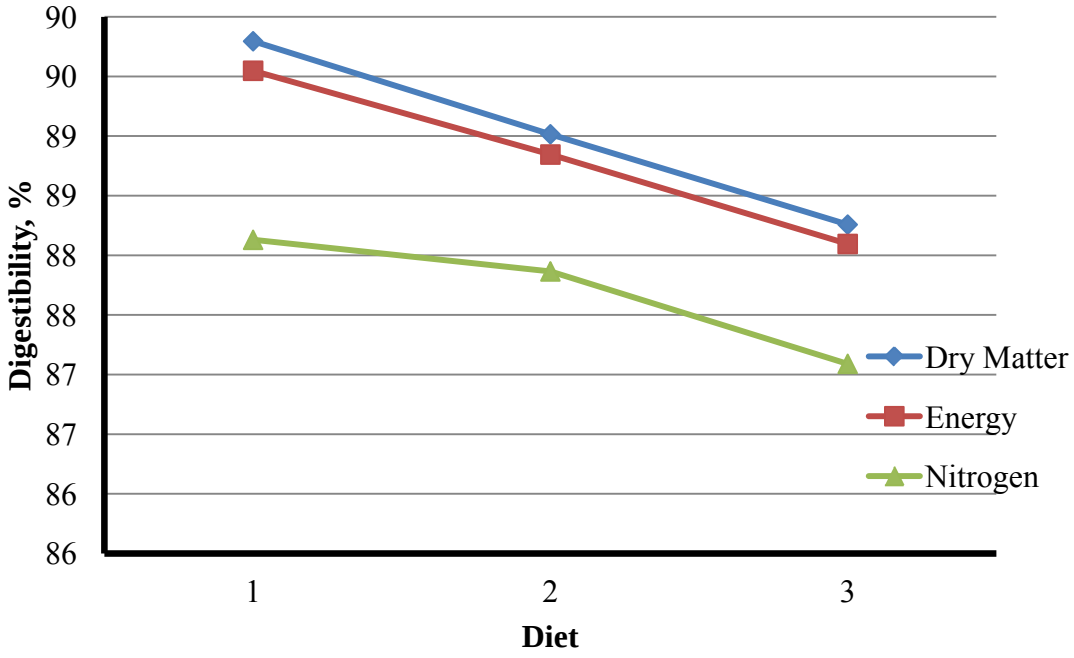


Figure 3.5. Period 5: Effect of naturally-contaminated corn on DM, energy, and nitrogen apparent digestibility. Each mean represent 4 pens with 2 pigs/pen. Experimental period length was 4 days and feed was provided depending on BW% (5% for Replicate 2, and 6% for Replicates 1, 3 and 4). No diet effects were observed ($P > 0.10$).

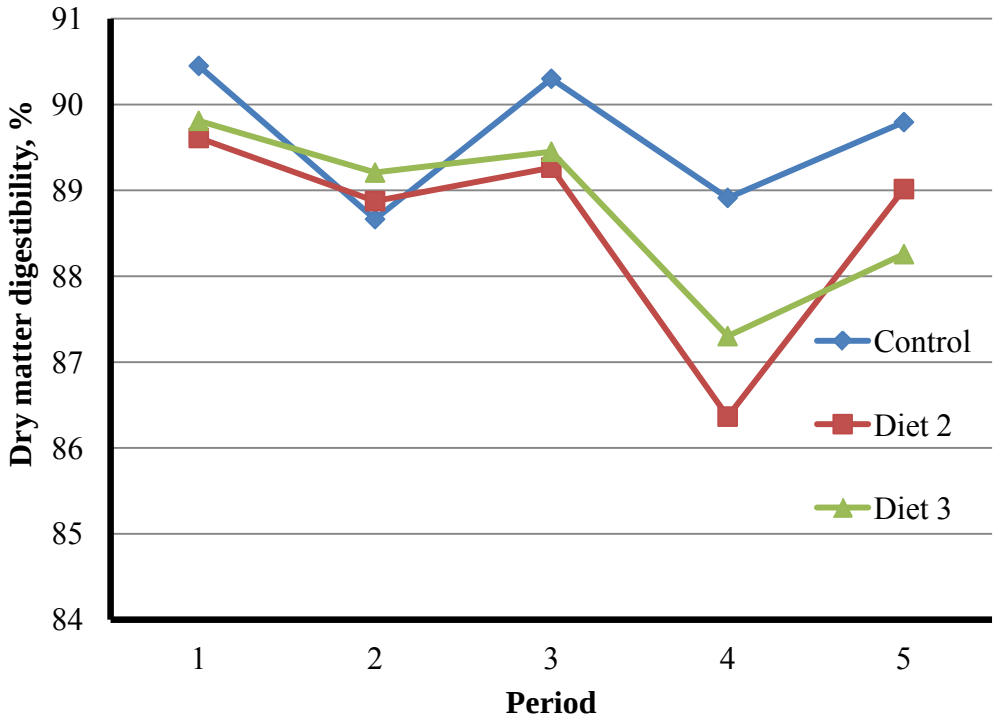


Figure 3.6. Periods 1-5: Effect of naturally-contaminated corn on apparent DM digestibility. Experimental period length was 4 days. Periods 4 and 5 were fed depending on BW% (5% for Replicate 2, and 6% for Replicates 1, 3 and 4). No diet effects were observed ($P > 0.10$).

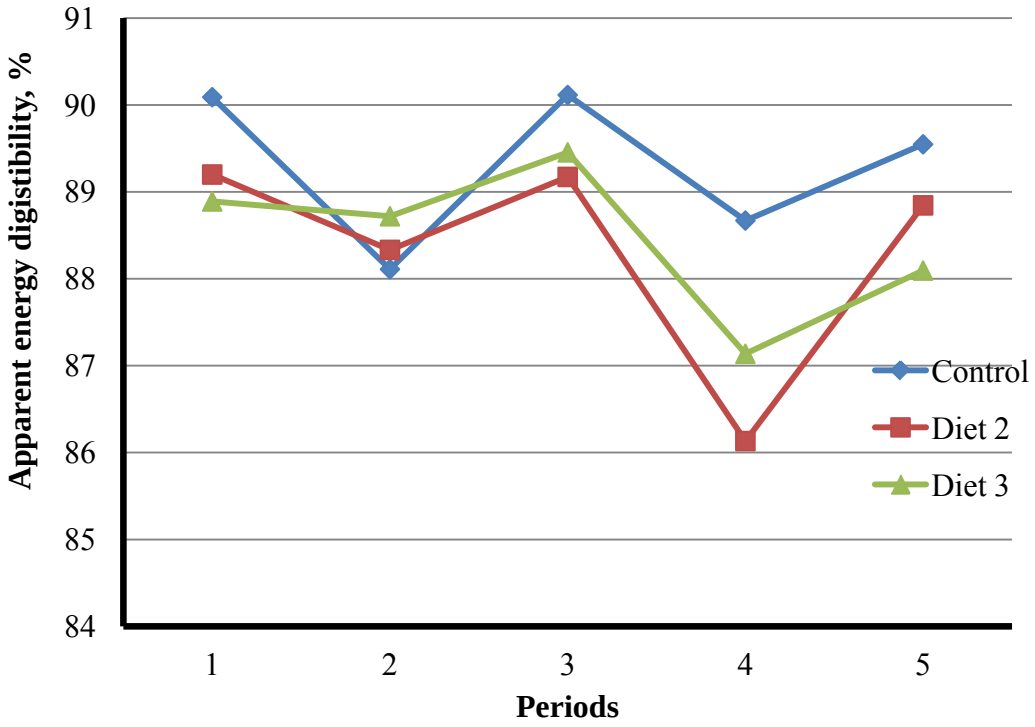


Figure 3.7. Periods 1-5: Effect of naturally-contaminated corn on apparent energy digestibility. Experimental period length was 4 days. Periods 4 and 5 were fed depending on BW% (5% for Replicate 2, and 6% for Replicates 1, 3 and 4). No diet effects were observed ($P > 0.10$).

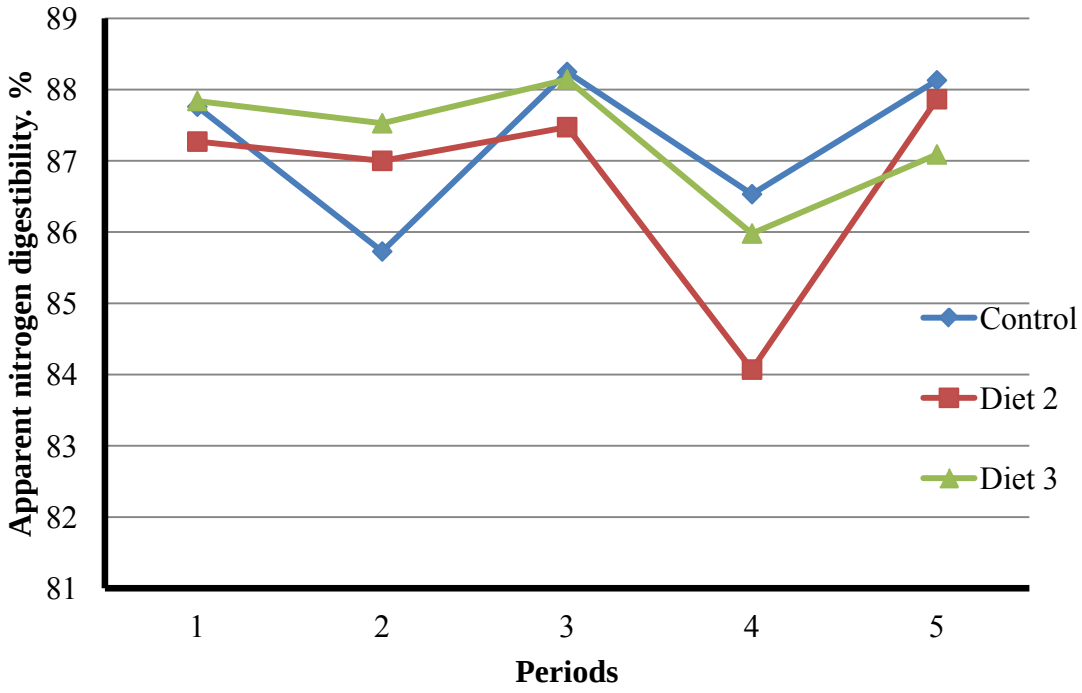


Figure 3.8. Periods 1-5: Effect of naturally-contaminated corn on a pparent nitrogen digestibility. Experimental period length was 4 days. Periods 4 and 5 were fed depending on BW% (5% for Replicate 2, and 6% for Replicates 1, 3 a nd 4). No diet effects were observed (P > 0.10).

3.3.2 Experiment 2

A preference (or an increase in feed intake) was shown for the naturally-contaminated 2010 corn diet over both the 50-50 % blend with 2010 and 2009, corn and the all 2009 corn diet (Table 3.10 and Table 3.11). The preference was exhibited in week 1 for both comparisons (Comparison 1: 88.55% vs. 11.45%; Comparison 2: 85.34% vs 14.66%; P < 0.001) and continued throughout the entire 3 wk period (Comparison 1: 96.61% vs. 3.39%; Comparison 2: 89.81% vs 10.19%; P < 0.001). Also, the accumulative preference for all periods showed the same pattern (Comparison 1: 95.34% vs. 4.66%; Comparison 2: 91.29% vs. 8.71%; P < 0.001).

Table 3.10. Effect of naturally-contaminated corn on feed preference .[1]

			Comparison (Control vs Diet 3)		
Period	Initial wt, kg	Final wt, kg	Control	Diet 3	*P*-value
1	8.15	10.66	88.55	11.45	< 0.01
2	10.66	14.51	97.60	2.40	< 0.01
3	14.51	19.23	96.61	3.39	< 0.01
1 - 3	8.15	19.23	95.34	4.66	< 0.01

[1]Each mean represent 3 pens with 5 pigs/pen. Diets were a complex nursery diet made with 2010 corn (control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). Experimental period length was 7 days. Fed *ad libitum*

Table 3.11. Effect of naturally-contaminated corn on feed preference.[1]

			Comparison (Control vs Diet 2)		
Period	Initial wt, kg	Final wt, kg	Control	Diet 3	*P*-value
1	7.82	10.49	85.34	14.66	< 0.01
2	10.49	14.05	96.54	3.46	< 0.01
3	14.05	18.64	89.81	10.19	< 0.01
1 - 3	7.82	18.64	91.29	8.71	< 0.01

[1]Each mean represent 3 pens with 5 pigs/pen. Diets were a complex nursery diet made with 2010 corn (control), the same diet formulation as the control replacing 100% of the corn with a 2009 mycotoxin naturally-contaminated corn (Diet 3), and a blend of 50% of the Control diet and 50% of Diet 3 (Diet 2). Experimental period length was 7 days. Fed *ad libitum*

3.4 Conclusions

3.4.1 Experiment 1

In this study it was shown that the exposure of naturally-contaminated corn in diets with levels ranging from 1.73 to 3.19 ppm of DON, 2.14 to 3.14 ppm of FB_1, and 0.7 to 1.40 ppm of ZEA, resulted in a dramatic decrease of feed intake, leading to low growth performance. Dry matter, energy, and nitrogen digestibility was not affected by either the 100% 2009 corn diet (Diet 3) or the 50% 2009 corn diet (Diet 2) compared to the 100% 2010 corn diet (Control), suggesting that these levels of DON, FB_1 and ZEA, and their possible synergistic interaction, are not affecting the digestibility performance of the weaning pig. This response is consistent with results from Danicke' et al. (2004) using

DON and Jiang' et al. (2010) using ZEA. Nevertheless, pigs consuming the 100% 2009 corn diet (Diet 3) showed a slight increase in digestibility compared to a less contaminated diet such as the 50% 2009 corn diet (Diet 2). This is probably a metabolic mechanism to help the reduced intake of nutrients due to the low feed intake.

3.4.2 Experiment 2

This experiment demonstrates the capability of weaning pigs to detect and choose a low naturally-contaminated corn (2010) over a more contaminated one (2009). If further demonstrates that exposure to naturally-contaminated corn in diet with levels ranging from 1.73 to 3.19 ppm of DON, 2.14 to 3.14 ppm of FB_1, and 0.7 to 1.40 ppm of ZEA is enough to create a preference in the pig. This responses are likely the result of either palatability or smell characteristics.

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VITA

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After completing his college and with his strong desire of pursuing masters in animal science, Carlos came to Lexington, Kentucky, and enrolled a master's program of swine nutrition in the college of Agriculture at the University of Kentucky under the supervision of Dr. Merlin D. Lindemann in January of 2010.