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An Assessment of Two Feed Additives to Improve Feed Utilization in Pigs

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AN ASSESSMENT OF TWO FEED ADDITIVES TO IMPROVE FEED UTILIZATION IN PIGS

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food, and Environment
at the University of Kentucky

By

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Lexington, Kentucky

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ABSTRACT OF THESIS

AN ASSESSMENT OF TWO FEED ADDITIVES TO IMPROVE FEED UTILIZATION IN PIGS

Three experiments were conducted to assess the efficacy of including selected feed additives in the diet of weaning and grow-finish pigs. Experiment 1 utilized 24 crossbred grow-finish pigs and measured the effect of added EHY on DM, N, and energy digestibility. There were no differences in DM, Energy, and N digestibility between diets 1 through 4. Experiment 2 utilized a total of 36 crossbred pigs [18 barrows, 18 gilts] in order to determine if preference would be shown when presented with naturally-contaminated corn. There were three dietary comparisons, Control vs Diet 2 (Comparison 1), Control vs Diet 4 (Comparison 2), and Diet 2 vs Diet 4 (Comparison 3). A preference was shown for the control diet over Diet 2, as well as for the control diet over Diet 4. Experiment 3 utilized a total of 24 crossbred pigs [12 barrows, 12 gilts] in order to measure the effect of contaminated corn on performance and DM, energy, and N digestibility. DM, energy, and N digestibility were affected by corn quality.

Keywords: EHY, Mycotoxins, Clay, Pigs, Preference

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CHAPTER 1. Introduction

The swine industry has evolved rapidly since the 1970s in an effort to produce pork economically and safely for the general public. There have been many improvements over the decades including confinement buildings, split sex feeding, genetic selection, and a sharp eye to diets that will produce pigs that grow efficiently, i.e. eating the least amount of feed per unit of growth. As feed costs rise due to increased competition for that feed, and while the demand for meat increases, it has become increasingly important to determine what diets and potential dietary additives will help keep the production of pork economical. The inefficiency associated with loss of dietary nutrients through feces and urine is a large concern as dietary nutrients like amino acids and minerals can be costly to add to diets, especially when the maximal digestibility has not been achieved. There has been an increase in research surrounding dietary additives other than antibiotics such as enzymatically hydrolyzed yeast as a way to increase nutrient digestibility and thus decrease nutrient losses and decrease the cost of adding additional nutrients to the diet.

Another concern to the swine industry is the prevalence of mycotoxin contaminated feeds. Mycotoxins are a concern to swine producers because swine are particularly susceptible to these toxins because they are found most commonly in the cereal grains from which swine diets are comprised. Mycotoxins are produced by fungi found on cereal grains and can occur while the grains are still in the fields as well as during harvest, post harvest, and in storage. Mycotoxins are often produced by fungi due

to weather conditions (drought, too much moisture, etc) and thus prevention of mycotoxin growth is next to impossible, making management of mycotoxin contaminated feed an important goal for the livestock industry. Binding agents such as clays (either as is from mines or after certain modifications) or various yeast or fermentation products are often added to contaminated diets in an effort to reduce the negative effects the toxins might have on the growth and health of swine. The objective of this research was to determine whether two selected feed additives would have an effect on nitrogen, energy, and dry matter digestibility in weanling or growing swine.

CHAPTER 2. Literature Review

2.1. Energy Utilization in Swine

Nutrient and energy utilization in swine are complex processes that are essential to the productivity of swine. The efficiency and profitability of production agriculture is affected by many variables but is largely a balancing act between cost of production and meeting animal needs. Producers must balance the many factors that go into decisions about feed rations, including cost, age/weight of pigs, area of the country, operation type, environment, health status, and availability of good feedstuffs (Pettigrew et al, 2001). Feed costs account for 65-70% of the money spent in pig production (Shelton et al, 2004; NSS, 1995). In order to illustrate the balancing act that is animal nutrition, then one should look at what is known about nutrient utilization in pigs. Consumers are looking for a lean product which increases the need for the producers to maximize leanness and minimize fat deposition in commercial pigs as fat deposition is very costly (Stalder et al, 1998**)**. As energy intake increases, protein accretion increases as well, until a plateau in growth is reached (Pettigrew et al, 2001**)**. Amino acids (AA) are an important part of swine diets that can be difficult to manage due to variability of feedstuffs. It is difficult to be sure of the nutritional values of feedstuffs due to the variation that arises from field and farm conditions, as well as processing and handling of the feed. The National Research Council (NRC, 2012) is the most reliable source to estimate protein and AA requirements.

Improper dietary formulation often leads to excess excretion of nutrients due to improper balancing for animal needs. Excess excretion of phosphorus (P) and nitrogen

(N) are of the largest concern to the environment as they are excreted in feces, which can then be used as fertilizer for cropland and has the potential to negatively impact the soil and local waterways if not properly applied. There have been many assessments of, and practices advocated, to help decrease excretion of P and N including genetic selection (Shirali et al, 2012**)**, phase feeding (Pettigrew et al, 2001), low-protein amino acid-supplemented diets (which may impair growth), and restricting feed intake of finishers (Warkup et al 1990; Ellis et al 1996; Blanchard et al 1999), to name a few. The balance is in producing a lean product without detracting from the eating quality and harming the environment (Pettigrew et al, 2001). It is impossible to improve nutrient utilization to 100% (or much above 50%) (Ferket et al, 2002). The ways in which N and P that are excreted can affect the environment and the methods utilized to decrease this impact will be discussed in later sections.

2.2.1. Nitrogen/Amino Acids

The amount of nitrogen in a feedstuff is used to determine the crude protein (CP) content of a feedstuff (Jurgens and Bregendahl, 2007). Nitrogen is an essential component of amino acids and constitutes, on average, about 16% of the crude protein. Nitrogen utilization depends on dietary availability as well as production status (Ferket et al, 2002). Amino acids are considered the building blocks of proteins, as well as be precursors of nitrogenous-based substances, used in creatine, dopamine, and catecholamines which are necessary to provide homeostasis in the body (Wu, 2010). There are 20 primary amino acids (Table 2.1), of which 10 are generally considered essential (must be obtained from diet due to inadequate or no synthesis) and 10 are

considered non-essential (can be synthesized in adequate amounts by animal). Amino acids are absorbed in the small intestine and occasionally fermented in the large intestine. One source of error in trials on nitrogen absorption and excretion in pigs is the fact that there are apparent ileal digestibility (AID) values and there are standardized ileal digestibility (SID) values which account for endogenous losses (NRC, 2012). Typically, for every 100g of protein consumed, a pig will secrete about 30g of endogenous protein into the digestive tract (Souffrant et al, 1993). It is assumed that 15% of N consumed by an animal is lost in feces and that 50% of consumed N is lost in the urine (Ferket et al, 2002). In general, fecal and urinary N excretion from poultry and swine account for about 65% of the N consumed, of which about 20% is lost to the atmosphere as volatized ammonia (van Heugten et al, 2000). Amino acids are generally obtained in the diet from cereal grains such as corn but because of the low percentage of certain amino acids (primarily lysine) supplied by cereal grains (30-60% of the total dietary AA), soybean meal (SBM) or synthetic amino acids are added to the diets as well (NRC, 2012). Corn and soybean meal are the most common components of pig diets in the United States as they are the most abundant grains and protein supplements and thus provide the best combination of nutrients for maintenance and growth in pigs at a reasonable cost.

Table 2.1. Amino Acids

Essential	Nonessential	Conditionally Essential
Histidine	Alanine	Arginine
Isoleucine	Asparagine	Cysteine
Leucine	Aspartate	Glutamine
Lysine	Glutamate	Proline
Methionine	Glycine	Tyrosine
Phenylalanine	Serine	
Threonine		
Tryptophan		
Valine		

Taken from NRC, 2012

2.2.2. Soybean Meal

Soybean meal is utilized because of its high protein content as well as available lysine, the rate limiting amino acid for growth in pigs. As of 2002, the American Soybean Association (ASA) stated that soybean meal was 62% of protein sources in animal diets (ASA, 2002). The United States supplies 42% of the world's soybeans, with Brazil supplying 24%, Argentina 16%, China 8% and India 3%. Factors such as crop care, rainfall, temperature, sunlight can all have an effect on the quality of the soybeans which in turn affects CP, total dietary fiber (TDF), and fat content. It has been theorized that as quality increases, so does AA concentration and protein solubility while TDF concentrations decrease. In order to complicate matters, just because the SBM composition is different in different countries does not necessarily mean there are digestibility differences. A joint project at the University of Illinois and The Ohio State University aimed to see how the soybeans and SBM from these five countries would compare. The experiment took high, medium, and low quality samples of soybeans and soybean meal from each country, then processed the soybeans from the outside countries

in the United States to compare processing methods and the effect on true AA digestibility (Karr-Lilienthal et al, 2004). The soybeans from every country that were processed into soybean meal had lower AA digestibility than the SBM originally processed in the individual countries, with the US being the exception. This could potentially show that SBM from other countries may be under-processed since heating is used to denature protease inhibitors in the soybeans which adversely affect the digestibility of SBM if not inactivated.

There have been many research projects performed to evaluate the efficacy of partially replacing SBM with other feedstuffs in order to cut feed costs but not reduce growth and carcass characteristics (Aherne and Kennelly, 1985; Thacker and Kirkwood, 1990). Shelton et al (2001) sought to determine the value of replacing SBM with the following feedstuffs: extruded beans, canola meal, peanut meal, sunflower meal, peas, meat and bone meal, and poultry-byproduct. Diets were formulated with only one source of protein per treatment. The research from Shelton et al (2001; Table 2.2) found that pigs fed a non-SBM diet experienced decreased average daily gain (ADG), decreased average daily feed intake (ADFI), and decreased lean gain. There was some variability in backfat deposition and gain:feed ratio but overall pigs fed SBM diets performed the best. It was noted that early growth was impacted the most in pigs fed non-SBM diets due to a higher protein requirement in younger pigs. It was determined that SBM remains the most efficient and highest quality protein source for pig diets due to the increased performance and carcass traits compared to the other protein sources. This may be due to the fact that many other protein sources contain anti-nutritional factors such as

glucosinolates (canola meal), tannins (canola meal, peanutmeal, peas), and protease inhibitors (peanutmeal, peas) which can all decrease growth and, ultimately, carcass values (Table 2.3; Shelton, 2001).

Table 2.2. Effect of protein sources on growth performance

	Protein Source								
Item	SBM	Corn-AA	ESB	Canola	Peanut	Sunflower	Peas	Meat-bone	Poultry
Grower period									
ADG, kg	0.81	0.56d	0.78	0.61d	0.75	0.62d	0.78	0.70d	0.69d
ADFI, kg	1.89	1.82	1.81	1.92	1.83	2.11d	2.12d	1.85	1.85
Gain: feed	0.43	0.31d	0.43	0.32d	0.41	0.30d	0.37d	0.38d	0.37d
Early-finisher period									
ADG, kg	0.93	0.77d	0.96	0.84	0.91	0.86	0.88	0.9	0.88
ADFI, kg	2.95	2.69	2.64	3.06	2.85	3.17	2.93	2.60e	2.61e
Gain: feed	0.32	0.29e	0.37d	0.28d	0.32	0.27d	0.3	0.35	0.34
Late-finisher period									
ADG, kg	0.84	0.85	0.79	0.86	0.86	0.81	0.81	0.74	0.75
ADFI, kg	3.25	3.06	2.91	3.35	3.14	3.69e	3.02	2.96	2.80e
Gain: feed	0.26	0.28	0.27	0.27	0.27	0.22d	0.27	0.25	0.27
Overall									
ADG, kg	0.85	0.70d	0.83	0.74d	0.82	0.76d	0.82	0.75d	0.77d
ADFI, kg	2.76	2.45d	2.47d	2.62	2.6	2.96	2.68	2.47d	2.41d
Gain: feed	0.31	0.28d	0.34d	0.29	0.32	0.26d	0.3	0.3	0.32
FBW, kg	120	110	118	112	118	119	114	113	115

Taken from Shelton, 2001

[a] Data are means of eight replicates of five pigs per replicate.Average intitial and final BW were 30.1 and 114.1kg, respectively. SBM=soybean meal; corn-AA= corn + crystalline AA; ESB=extruded soybeans; canola=canola meal; peanut=peanut meal; sunflower=sunflower meal; pea=ground peas; meat and bone=meat and bone meal; poultry=poultry by-product meal; FBW=final body weight.

[b] LSD = least significant difference (P < 0.05)

[c] overall treatment effect

[d] Significant difference compared with SBM (P<0.05)

[e] Significant difference compared with SBM (P<0.10)

Table 2.3. Effect of protein sources on carcass characteristics

	Protein Source								
Item	SBM	Corn-AA	ESB	Canola	Peanut	Sunflower	Peas	Meat-bone	Poultry
Loin muscle area, cm2	43.29	31.87d	42.2	41.79	41.63	42.2	41.55	40.47d	43.74
10th-rib backfat, cm	1.87	2.67d	2.05	1.98	1.99	1.94	2.10e	2.15d	2.06
Average backfat, cm	2.6	3.01d	2.89d	2.81e	2.76	2.55	2.78	2.87d	2.83d
Carcass length, cm	84.18	84.53	84.12	83.02	84.2	84.22	84.01	83.75	83.6
Dressing percentage	74.69	73.60e	75.23	74.74	75.33	73.86	75.09	75.3	75.51
NPPC aceptable quality lean, %	53.93	47.06d	52.89	53.15	52.93	53.53	52.51e	51.98d	53.32
NPPC lean, kg	46.52	40.26d	45.9	45.76	45.98	45.61	45.17d	45.16d	46.46
TOBEC									
Fat, kg	25.04	32.14d	27.73d	26.46	26.83	25.2	26.98	28.66d	27.54e
Fat, %	28.67	37d	31.07	29.89	29.93	28.86	31.88d	32.04d	30.74
Fat-free lean, kg	43.69	37.64d	42.58	42.83	44.67	45.35	41.26e	41.95	42.26
Fat-free lean, %	49.89	43.39d	47.83	48.54	50.32	51.59	48.11	47.10e	47.55
Lean gain per day, g	284	2.14d	267	262	274	272	247d	254d	260
Lean:fat	1.84	1.18d	1.57e	1.65	1.72	1.83	1.56e	1.50d	1.58e

Taken from Shelton, 2001

[a] Data are means of four replicates of 10 to 12 pigs per replicate. SBM = soybean meal; corn-AA=corn + crystalline AA; ESB= extruded soybean meal; sunflower = sunflower meal; pea= ground peas; meat and bone = meat and bone meal; poultry= poultry by-product meal.

[b] LSD = least significant difference (P<0.05)

[c] overall treatment effect

[d] Significant difference compared with SBM (P<0.05)

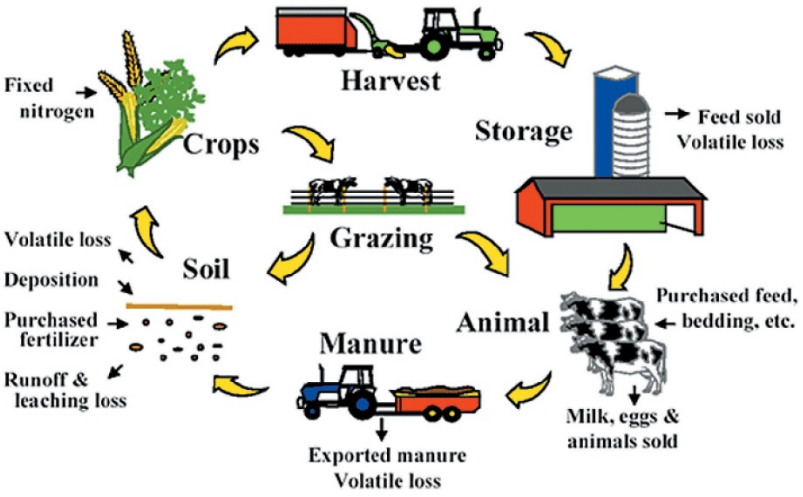
[e] Significant difference compared with SBM (P<0.10)

It is important to note that processing and handling of feedstuffs can change the nutritional values and can counteract various anti-nutritional factors. With ethanol production there has been an increase in ethanol byproducts. The most common byproduct, dried distillers grains with solubles (DDGS), is fed most often to cattle because it is relatively cheap and has high CP (Singh et al, 1998; Jacela et al, 2008; Saunders et al, 2009). Pigs are not as efficient at utilizing DDGS as ruminants because they lack the microbial fermentation to break down the germ of the plant which contains the most phytate P. Fractionation, removal of the endosperm, germ, and bran of corn before fermentation leads to a more efficient use of starch in the animal. Because the bran and germ are removed, the CP of the byproduct is increased while the fat and fiber contents are decreased, leaving one with dried distillers grains (DDG) (Murthy et al,

2006). The ways in which feeds are processed can also affect nutrient content of that feed. Often DDG is dried with steam instead of direct heat, causing less change to the feed. Sorghum has a greater CP percentage than corn. Jacela et al (2010) used 22.7kg barrows on three diets: 67% HPC-DDG, 50% HPS-DDG, and a N-free diet to determine the AA digestibility and calculated energy values of HPC-DDG and HPS-DDGS. It was found that HPC contained a CP of 40.8%, lysine at 1.36%, and the lysine-CP ratio was 3.2% (want better than 2.8% in pig diets-Jacela et al, 2010). In the sorghum, CP was 48.2% and most AA were in greater proportions than HPC. However, while the sorghum had a better AA profile and greater AA concentrations, it also had lower digestibility and energy (Jacela et al, 2010). Ultimately, distillers grains can be used in swine diets but are not as nutrient rich SBM.

2.2.3. Nitrogen Excretion & Management

Figure 2.1. The Nutrient Cycle of Nitrogen



Taken from Rotz, 2004

There is significant concern over nitrogen excretion in pigs because there is potential for N loss from manure management and contamination of groundwater and environmental pollution when manure is utilized as fertilizer in crop fields. Nitrogen loss occurs mostly through volatile losses into the atmosphere and leaching/runoff losses into ground and surface waters. Nitrate leaching into groundwater has been a major concern. Nitrification and denitrification emit nitrous oxide into atmosphere and ammonia emissions affect fertilization, acidification and eutrophication in ecosystems. Nitrous oxide is a concern for global warming (Rotz, 2004). There are essentially two parts to manure management: the actual storage of the manure and the field application of the manure as fertilizer. Manure is stored in the following ways: as solids, slurry, or liquid with dry matters of >15%, 7-15%, and <7%. Loss of N from manure is affected by DM content, total N concentration, ammoniacal N concentration and pH. Low losses of N occur below a pH of 6 and high losses occur when the pH of a pit is above 8 (Muck and Steenhuis, 1982). Environmental factors affecting the loss of N in stored manure include ambient temperature, wind, solar radiation, as well as the type of manure storage whether it be an open lagoon, a series of lagoons, or anaerobic pits (Table 2.4 and Table 2.5) (Sommer et al, 1997). It has been shown that lagoons have a higher N_2O emission than slurry storage. Low temperatures often suppress microbial activities which accounts for an increase in CH_4 emissions in the warmer months (Liu et al, 2013). The type of barn has also been said to contribute to the amount of N found in lagoons. In an evaluation of hoop barns by DeRouchey et al (2002), it was found that finishing and wean-to-finish barns had greater concentrations of total N than sow and farrow-to-finish barns. P and Ca

levels in farrow-to-finish lagoons were lower than all of the other operations though as well as containing the lowest trace mineral contents (Cu, Zn, Fe, Mn). With that said though, there is a large amount of variation between operations with regards to the composition of the manure pits. Overall it was seen that ammonia, total N and organic N were lowest in the coldest months (December to February) and highest in the warmest months (June and August) due to an increased activity of bacteria causing the volatilization of NH_3. It has also been concluded that the higher surface/volume ratio manure pits have lower nutrient concentrations. DeRouchey et al (2002) reported that the average N concentration published in 1993 by the Mid-West Service was 625ppm while the average P concentration in manure pits was 165ppm.

Table 2.4. Typical N Losses from Animal Housing Facilities Expressed as A Percentage of total N Excreted

Manure Type	Typical loss, % total N	Range, % total N	N form lost
Swine, slatted floor	25	15 to 30	NH_3
Swine, deep litter	50	50 to 60	NH_3, N_2O, N_2
Swine, free range	35	25 to 40	NH_3, NO_3, N_2

Adapted from Rotz, 2004

Table 2.5. Typical N Losses for the Major Types of Long-term Manure Storage Used in Animal Production Expressed as a Percentage of Total N Entering Storage

Manure Type	DM content, %	Typical loss, % total N	Range, % total N	N form lost
Solid compost	40	40	20 to 50	NH_3, NO_3
Slurry tank, top	10	30	20 to 35	NH_3
Slurry tank, bottom	10	8	5 to 10	NH_3
Slurry tank	10	4	2 to 8	NH_3
Anaerobic lagoon	5	70	50 to 99	NH_3, N_2O, N_2

Adapted from Rotz, 2004

Each farm must be looked at individually to determine the best course of action for manure management. There are susbstances that can be added to manure pits in order to reduce the pH as a way to cut down on ammonia volatilization. These additives include acids, base precipitating salts and labile carbon, but each has their own pros and cons. Acids tend to be expensive, very corrosive and potentially hazardous to the health of the animals and the human workers. Salts successfully reduce the pH of a pit but are not effective at sustaining low pH levels. Carbon treatments such as sucrose and potato starch can see 42-98% reductions in volatilization by stimulating anaerobic microorganisms to produce organic acids but large quantities are needed so it is not economical and losses following application are also seen (Rotz, 2004).

The second part of nitrogen management is the application of the manure to cropland. The nitrogen needs to be applied in large amounts within a short time (rapid incorporation into the soil) just before seeding of a plant or while the plant is actively growing to be used most efficiently. Higher application rates reduce the loss of N but

may not always be feasible. Low dry matter (slurry) is absorbed more rapidly into the soil but the increase in manure volume can also increase the chance of accidental loss on roads during transport. Ideally a pH below 7 before application should be achieved (Sommer and Hutchings, 1995). The application type also determines the amount of nitrogenous loss (Table 2.6). The scientific standpoint is not the only one that counts though as there are regulations within states, counties and even towns that can dictate how and when farmers may apply manure as fertilizer.

Table 2.6. Typical N Losses for Major Manure Application Methods Expressed as a Percentage of the Initial Total N Applied

	Ammonia Loss		**Other N Loss**	
Manure Type	**Average**	**Range**	**NO_2**	**N_2O**
	% total N			
Irrigated slurry	30	25 to 50	2 to 25	<1 to 4
Broadcast slurry on grassland	25	15 to 40	1 to 25	<1 to 4
Broadcast slurry on bare soil	20	10 to 27	1 to 25	<1 to 4
Broadcast of solid cattle or swine	20	8 to 60	1 to 25	<1 to 4
Broadcast of solid poultry	12	8 to 25	1 to 25	<1 to 4
Band or trailing hose of slurry	18	13 to 26	1 to 25	<1 to 4
Incorporated within 6 hours	10	6 to 13	1 to 25	<1 to 4
Shallow injection of slurry	8	7 to 12	2 to 25	<1 to 4
Deep injection of slurry	2	1 to 5	5 to 25	2 to 9
Grazing feces and urine	10	4 to 20	10 to 30	<1 to 8
Taken from Rotz, 2004				

When it comes to reducing N excretion there are several options laid out. One of these options includes multiphase feeding (preferably 3 phases or more in the grow-finish period) which allows animals to be fed closer to their changing bodily needs as well as

using separate diets for gestating or lactating sows. It has also been found that reducing feed particle size improves nutrient digestibility by increasing surface area and thereby allowing more access to enzymes in the gastrointestinal tract (Han et al, 2001). Addition of synthetic AAs combined with a decrease in dietary CP is also useful. Ideal protein in pigs varies by sex, age, genotype and production function. It is estimated that a 1% reduction in CP content of pig diets could reduce N excretion by 8% (Kerr et al, 2003; NRC, 2012). CP can be reduced by about 2-4% without detrimental effects to carcasses by appropriately supplementing synthetic AAs (Han and Lee, 2000). Nitrogen excretion is largely dependent upon genetics, gender, diet, housing system, body weight, as well as age.

It has also been found that the skeletal ryanodine receptor 1 (RYR1) gene found in Pietrain pigs and their offspring has an influence on feed efficiency and carcass characteristics (Shirali et al, 2012). It was found that NN (homozygous normal) pigs had lower nitrogen excretion than Nn (heterozygous carrier) pigs from 60-140kg. The most important thing to retain from this research is that nutrient needs are constantly changing in pigs. Nitrogen efficiency also decreases as pigs grow, with the lower weight pigs (up to 90kg) having more efficient retention than larger finishing pigs or adult swine. The results showed 32% retention and 68% excretion which reinforce the idea that nitrogen efficiency in pigs decreases as they grow.

2.3.1. Phosphorus

Phosphorus (in conjunction with calcium) plays an important role in the skeletal system. And, as is common with other nutrients, there are many different requirement estimates depending upon age, sex, production status, and genetics. It is commonly held that gilts require more P than barrows, but boars require an even larger amount of P (NRC, 2012). About 60-75% (up to 85%, Akinmusire et al, 2009) of P is bound as phytate in cereal grains and oilseed meals (canola and soybean to name a few), making the P biologically unavailable to pigs consuming diets based on these feedstuffs. The range of availability is less than 15% in corn to greater than 50% in wheat (which has a naturally occurring phytase enzyme) (NRC, 2012). Historically, in order to meet the P need of animals, diets were supplemented using inorganic P in the form of rock phosphates (estimated use of 148 million tons per year) (Kebreab et al, 2011). This is an unsustainable practice as eventually this source of nonrenewable inorganic P will be played out. Additionally, excess excretion of P (unused phytate P) creates environmental issues. The most successful way of creating more available P to pigs has been the addition of phytase to the diet. Phytase is an enzyme that frees orthophosphates and inositol from phytate, essentially it allows for the release of some P molecules from the phytic acid, making P more digestible and available to the pigs (Kebreab et al, 2011).

The concern for environmental effects of concentrated pig farming is the increase in manure, odor, ammonia, potential for nutrient runoff, and greenhouse gas emission. Traditionally manure was applied to cropland in order to meet N needs, but now it is more complicated. Application rates or limits are often determined by both N and P

content and needs of the soil/cropland to which the manure is being applied. Excess N and P can both do serious damage to water, air, etc (Hinson et al, 2009). Decreasing P excretion is important because runoff from fields can affect surface water and ecosystems. When P is overloaded into a water source there is a spike in plant growth and an increase in plant type, changes in pH, and depletion of oxygen in the water. It has been reported that pigs and poultry produce 20% of the manure from animal production but excrete 36% of the total P, which again shows how inefficient pigs are at digesting nutrients (Ferket et al, 2002).

2.4.1. Environmental Impacts of Nitrogen and Phosphorus

Peterson (2010) breaks down the environmental impacts of pigs into four headings: manure management, manure storage and handling, air quality, and soil nutrient levels. Manure management refers to the care of manure in such a way that nutrients necessary for crop production are retained optimally within the stored manure. Unfortunately the balance of nutrients found in pig manure is not optimal for crop growth. Adjustments to diets that reduce outrun (reduction of nutrient waste and decrease N and P that end up in the soil/water) are part of the solution at hand. Swine manure is 60:40 available N to unavailable organic N. Manure may reach N needs but be in excess of P (increased algae growth, groundwater contamination of P). Manure storage and handling is important. Increased solids decrease uniform application to land and increases potential for N volatilization, but pit additives can be added to improve digestion of the manure. The amount of ammonia is considered an air quality concern with regards to pig

manure. Ammonia, which occurs due to the volatilization of N negatively affects humans and pigs by decreasing performance and increasing respiratory problems. With regards to soil nutrients, mineral concentrations in manure are 10 times the concentration found in feed which can lead to imbalances in soil and crops when manure is applied as a fertilizer. The European Union, Colorado and South Carolina have all begun to test for Zn levels in soil as well as N and P in order to reduce potential toxicity issues due to increased trace mineral levels in soil (Peterson, 2010). White (2010) lists air and water quality, human health, and pathogens associated with manure as causes for concern. Waste, inorganic fertilizers, nitrates, phosphorus, trace minerals, microbes, antibiotics, pharmaceuticals, odors, dusts, bacteria, viruses, and parasites in manure are all considered environmental pollutants.

It has been mentioned before that production facilities have an impact on the environment and that producers and scientists are working to reduce the impact that occurs. Lammers et al (2012) performed Life-cycle assessments (LCA) which are the environmental impacts that occur from products or processes. Factors that affect these include source/type of feed, dietary strategies, climate, size of the operation as well as a number of other management strategies. These vary greatly between Europe and the United States, as well as regionally within each entity. It was estimated that in farrow-to-finish operations, group housed sows produced 7% more live pigs than gestation stalled sows. Pigs in hoop barns require 8% more feed during the winter months than pigs raised in conventional buildings and also had 7% smaller sow herds. The difference between the two types of operations (conventional versus hoop barns) is that sows in the conventional

system are housed on concrete and buildings utilize mechanical ventilation while sows in hoop barns live on floors covered in corn stalks and have natural ventilation (Lammers et al, 2012).

Obviously a large concern for manure quality is that nutrients are lost when the manure is stored and then when applied dependent upon weather conditions (Lammers et al, 2012). Nitrogen losses have been said to be 35-45% from cornstalk hoop barns and 28% in straw deep-litter pens (but up to 75%). Because P does not volatilize, it is assumed that all the P makes it to the field, but it is important to meet both the N and P requirements of the field. It was assumed that the conventional systems lost less N than hoop barns (25% vs 50%).

2.5.1. Yeast Products

Feed additives are designed to improve growth and feed efficiency. To the extent that they do this, they also reduce nutrient excretion. An early examination indicated that the addition of yeast products to swine diets did not improve performance (Loeffel et al, 1937) but the addition of antibiotics to diets has been shown for years to improve growth performance. A large concern presently is the regulations involving antibiotic addition to feeds, antibiotic bans in some countries require other sources to help improve performance in swine. Antibiotic growth promoters (AGP) have been banned in the European Union and some are currently being voluntarily removed from the market in the United States, requiring other additives to be studied and potentially marketed as growth promoting. In more recent work, Bowman and Veum (1973) also found that

supplementation of 1.5 or 2.0% SCYC (*saccharomyces cervisiae* yeast culture) in growing (14 to 34 kg) and finishing (34 to 100 kg) diets did not affect the growth performance or carcass characteristics when compared with pigs on a normal diet. While Kornegay et al (1995) found that the addition of yeast culture to corn-SBM starter diets had no effect on performance of the weanling pigs, the yeast was more active and showed more performance differences in pigs fed different fiber types (i.e. soybean hulls or peanut hulls). In other studies, yeast cultures have been demonstrated to improve nutrient digestibility of weaning pigs (Shen et al, 2009), as well as promoting feed intake (Bowman and Veum, 1973; Shen et al., 2009), increasing ADG and strengthening the immune system (Shen et al., 2009). Fermentation products of *S. cerevisiae* have been shown to improve litter performance when fed to breeding and gestating sows (Shen, 2011). It has also been shown that the use of *S. cerevisiae* does not alter the enteric microflora, specifically the ileum, of swine, as it has been shown to do in cattle (Mathew et al, 1998). It has been hypothesized that different strains of the yeast may have differing properties and thus affect the microflora differently. The use of *S. cerevisiae* fermentation products has been shown to enhance intestinal morphology and thus allow pigs infected with *Salmonella* to have greater digestive capacity and show enhanced growth performance (Price et al, 2010).

2.6.1. Mycotoxin Background

Mycotoxins are the toxic byproducts of mold infestations in crops. There are five generally accepted primary groups of mycotoxins that include aflatoxins, vomitoxins, ochratoxin A, fumonisin, and zearalenone. Mycotoxins are a concern in crops because of

the lost profit they represent but more importantly because of the potential health risks associated with ingestion (ERS, 2011). The Food and Agriculture Organization (FAO) states that at least 25% of the world's crops are affected by mycotoxins which makes mycotoxins a potential concern (USDA GIPSA, 2006). CAST (2003) found that crop loss in corn, wheat, and peanuts from mycotoxin contamination in the United States cost $932 million along with $466 million in regulatory measures each year, testing procedures and quality control. Mycotoxins affect everyone that may be involved with grain, including the grain producer, middlemen, and ultimately the consumer (Table 2.7). Each step sees limited yields, restricted markets, and an effect on the price of the grain. There is ultimately the concern of an end market for mycotoxin-infected grains because it may not pass inspection levels in order to be used in human food or animal feed (USDA GIPSA, 2006).

Table 2.7. The Cost of Mycotoxins

Producer Costs	**Handler/Distributor costs**
Crops	Extra drying costs
Yield losses	Excess storage capacity
Restricted markets	Losses in transit
Nonmarketable product	Loss of markets
Price discounts	
Increased production costs	**Processor costs**
Pest control	Milled corn products
Irrigation	Restricted markets
Increased postharvest costs	Product loss
On-farm drying	Peanut products
On-farm testing and sampling	Insurance premiums
On-farm detoxification	Restricted markets
Increased transportation costs	Product loss
Inability to obtain loans on stored grai	Fermentation products
Disposal of useless crops	
	Consumer costs
Livestock producers	Less nutritious food
Higher mortality rates	Higher product prices
Reproductive failures	Long term chronic effects from low-level contamination
Reduced feed efficiency	
Higher feed costs	**Social Costs**
Lower live weight	Regulatory costs
Infertility syndrome	Establishing standards and tolerances
Increased susceptibility to disease	Surveillance and assay
Overall quality loss	Enforcement
Monitoring and testing	Research and extension
	Education
	Lower foreign exchange earnings
	Increased costs of imports
Taken from CAST-Mycotoxins: Economic and Health Risks (1989)	

The fungi can produce mycotoxins while the crop is in the field or during storage of the grains after harvest (Table 2.8). Temperature stress in the field, and high moisture and temperature during storage, are the acknowledged causes of fungi growth (ERS, 2011). Stress to the plant in the form of drought, flooding, and insect insurgency can also be responsible for mold growth and subsequent mycotoxin production. The mold robs nutrients from the grains it infects, and can change the color, texture and odor of the plant

itself. When feed spoils, the temperature increases which increases mold growth, resulting in reduced palatability and loss of nutritive value (Christensen et al, 1974). Mycotoxins are linked to birth defects, nervous system issues, tumors, and many other issues. Factors that can affect the severity of mycotoxicoses in those who ingest mycotoxins include health, age (young and old suffer effects more), sex (females are more susceptible to mycotoxicoses), environment, food storage, exposure level and duration, and lack of regulation/monitoring (USDA GIPSA, 2006).

Table 2.8. Mycotoxins Before and After Grain Harvest

Cereals	Pre-harvest	Post-harvest
Barley	DON, NIV, Zea, HT-2, T-2	OTA, Afla, Cit
Maize	DON, Fum, Zea	Zea, Afla
Oats	DON, NIV, HT-2, T-2	OTA, Cit
Rice		Afla, Sterig, OTA
Rye	Ergot	OTA
Sorghum	Ergot	Afla
Wheat	DON, NIV, Zea, ergot	OTA, Afla, Cit

(adapted from Petterrson, 2004)

Afla = aflatoxins; Cit = citrinin; DON = deoxynivalenol; Ergot = ergotamine; HT-2 = HT-2 toxin; NIV = nivalenol; OTA = ochratoxin A; Sterig = sterigmatocystin; Zea = zearalenone

The Food and Drug Administration (FDA) sets advisory, action, and regulatory levels with regards to mycotoxin contamination. Advisory levels are considered guidances and exist only for deoxynivalenol. Action levels (Table 2.9) are a precise level of contamination at which an agency may take regulatory action and exist for aflatoxins. The regulatory level is supposed to set exact established limits but there are currently no established limits for any contamination. Of most concern for swine are the levels of

vomitoxin (5ppm), fumonisins (in corn-20ppm and 10ppm maximum in the total diet), and aflatoxins (20ppb in immature animals and 200ppb in finishing swine) (FDA, 2011).

Table 2.9. FDA Action Levels For Aflatoxins

Species	Commodity	Action Level
Humans	Milk	0.5ppb
Humans	Any food except milk	20 ppb
Immature animals, dairy animals, or when end use is not known.	Corn or other grains	20 ppb
All species	Animal feed other than corn or cottonseed meal	20ppb
Breeding cattle, breeding swine, or mature poultry	Corn and other grains	100 ppb
Finishing swine of 100lbs or greater	Corn and other grains	200 ppb
Finishing beef cattle	Corn and other grains	300 ppb
Beef cattle, swine, poultry	Cottonseed meal	300 ppb

Taken from GIPSA, 2006

2.6.2. Mycotoxin Effects

Mycotoxins in animal feed are a concern due to the physiological effects from ingestion of mycotoxin contaminated feed and, ultimately, the economic losses. Swine and poultry are particularly susceptible to mycotoxins and focus in research has been to find ways to mitigate the damage with feed additives designed to bind to mycotoxins and reduce mycotoxicoses. The cost of mycotoxins is to animal health (Table 2.10) and ultimately a decrease in productive capabilities of livestock. Contamination leads to reduced feed intake, feed refusals, poor feed conversion and ultimately diminished body weight gain (Pearce, 2011). The economic impact is often difficult to calculate because the level and variety of contamination varies widely, as do prices for grains and feed

products, and the costs associated with mitigating contamination varies by operation (Bryden, 2012).

Table 2.10. Common mycotoxins, commodity affected, and health effects

Mycotoxin	Commodities	Fungal source(s)	Effects of ingestion
Aflatoxin B_1, B_2	Corn, peanuts, and many	*Aspergillus flavus*	
G_1, G_2	other commodities	*Aspergillus parasiticus*	Aflatoxin B1 identified as potent human carcinogen by IARC. Risk of human toxicosis. Adverse effects in various animals.
Deoxynivalenol	Wheat, corn, and barley	*Fusarium graminearum*	Human toxicoses in India, China, Japan, and Korea. Toxic to animals, especially pigs.
Nivalenol		*Fusarium crookwellense*	
(Vomitoxin)		*Fusarium culmorum*	
Zearalenone	Corn, wheat	*Fusarium graminearum*	Identified by the IARC as a possible carcinogen. Affects reproductive system in lab animals and pigs
		Fusarium culmorum	
		Fusarium crookwellense	
Ochratoxin A	Barley, wheat, and many	*Aspergillus ochraceus*	Suspected by IARC as human carcinogen.
	other commodities	*Penicillium verrucosum*	Carcinogenic in lab animals and pigs.
Fumonisin B_1	Corn	*Fusarium moniliforme*	Suspected by IARC as human carcinogen.
			Toxic to pigs and poultry. Cause of equine
			eucoencephalomalaic (ELEM), fatal to horses

Taken from ERS/USDA, 2011

Aflatoxins

Aflatoxins, produced by the *Aspergillus* species, are the most widely recognized mycotoxin because they are considered carcinogenic and attack the liver and immune system, which makes them a severe health risk to animals and humans. Natural contamination occurs in cereal grains, their by-products and oilseed meals. The most toxic aflatoxins are the AFB_1 and AFM_{10}. The primary concerns regarding aflatoxin contaminated feed are the carcinogenic, teratogenic, and mutagenic effects that occur in livestock. These toxins affect the liver, kidney and brain primarily but are also

immunosuppressants and impair reproduction (Gimeno, 2006). When 15-20kg pigs were fed 400-800ppb of AFB1 for a period of 3-9 months, a reduction in growth, damage to the liver and increased susceptibility to salmonellosis was observed (Edds, 1979).

Ochratoxins

Ochratoxins are also produced by the *Aspergillus* species of fungi, with Ochratoxin A (OTA) being the most toxic. Ochratoxins primarily affect the kidneys of animals that ingest them. In 20-90kg pigs fed 200-400ppb of Ochratoxins for 3-4 months, delayed growth and increased water intake were recorded (Carlton and Krogh, 1979). Immunosuppression was experienced in 20kg gilts when fed 2500ppb of OTA (Harvey et al, 1992).

Fusariums

There are several natural contaminants that are part of the *Fusarium* species and are most commonly contaminated in the field. Zearalenone (ZEN) mimics estrogen production which causes confusion for the reproductive systems, feminizing males and often causing infertility in females (USDA GIPSA, 2006). ZEN is a major concern in pre-pubescent and young sows as ZEN is an estrogenic compound. Because ZEN is estrogenic it inhibits follicle development and ovulation, a concern because it inhibits reproduction in swine and without sows that produce piglets, profit is not being earned (Gimeno, 2006). In 27-31kg pigs fed 1000 and 5000 ppb ZEN severe vulvovaginitis was witnessed (Mirocha and Christensen, 1974). Fumonisin B_1 and Fumonisin B_2 are

neurotoxic, nephrotoxic, hepatotoxic, and cause pulmonary, cerebral, and cardiac lesions (Gimeno, 2006). Fumonisin B_1 is more severe in barrows than gilts (Rotter et al, 1996).

Trichothecenes

The most common Trichothecene toxin is deoxynivalenol (also known as DON or vomitoxin) which, as the name implies, commonly causes gastroenteric problems, vomiting, as well as irregular heartbeats and diarrhea (Gimeno, 2006). Vomitoxin is most associated with feed refusal in pigs (Bryden, 2012). When fed DON at a rate of 300-700 ppb there was rejection of feed, vomiting, and decreased weight gain (Trenholm et al, 1983), as well as when DON was found in feed at higher levels (Bergsjo et al, 1993). *Fusarium* in corn and wheat damages the kernels which leads to production of deoxynivalenol (DON) which causes necrosis of the digestive tract and also causes damage to the reproductive systems.

Interactions

A major concern when dealing with mycotoxins is the fact that they rarely occur one at a time but often occur two or more at a time in feedstuffs. A research project by Harvey et al (1989) effectively shows the effect of combining mycotoxins. Six week old pigs were fed four different diets, 0 mg of DON and AF (control), 2.5 mg of DON/kg of feed, 0.75 mg of AF/kg of feed, and 2.5 mg of DON + 0.75 mg of AF/kg of feed. The pigs that ate the aflatoxin contaminated feed as well as the aflatoxin and DON diet saw decreases in body weight while pigs that ate DON and the combination diet saw vomiting and feed rejection. Mirocha et al (1978) also saw concerning evidence when feeding

multiple mycotoxins. Feeding the combination of DON and ZEN resulted in feed rejection, vomiting and bloody feces that were not observed when the toxins were fed individually.

2.6.3. Mitigating the Damage of Mycotoxins

There are several methods of mitigating the damage related to mycotoxin contamination. Prevention of mycotoxin growth while the crops are still in the field would be an ideal management method but unfortunately it is impossible to control the weather which creates the conditions that produce the mold growth and ultimately the production of mycotoxins. Because it can be difficult to prevent mold growth, feed additives are often the best form of management in order to prevent decreases in growth when livestock are fed high levels of contaminated feed (Bryden, 2012). Other options are discarding grains and mechanical sorting of contaminated grains (Muller, 1982), extraction of contaminants by organic solvents (Muller, 1982), and less productive because mycotoxins are heat stable would be the use of irradiation by UV light (Neely and West, 1972). The most common additives include adsorbents, preservative blends, and yeast products. Adsorbents are the easiest and cheapest to use, but are usually non-specific and bind to vitamins and nutrients along with the toxins and may have to be added to the diet in large amounts (Biomin, 2013). Yeast products derived from *S. cerevisiae* work to bind toxins and prevent absorption in the gastrointestinal tract (Patience et al, 2014). Solutions to contamination include activated charcoal which adsorbs mycotoxins (Huwing et al, 2001) but is ultimately largely excreted in the feces

(Buck and Bratich, 1986), yeast and probiotics to bind and transform mycotoxins (Pearce et al, 2011), and zeolites with specific electrical charge ability (Grubner et al, 1968).

Clays are the simplest additive used to prevent adverse effects associated with mycotoxin contaminated grains. The disadvantages of feeding contaminated feed to livestock includes reduced feed intake, lowered daily gains, and reduced feed efficiency (Harvey et al, 1988, Lindemann et al, 1988). Lindemann et al (1990) stated that up to 85% of performance losses due to aflatoxins have been recovered by the addition of 0.5% clay to the contaminated diets. Pigs fed aflatoxin-contaminated diets containing clay consumed more and grew faster ($P < 0.01$) than pigs fed contaminated diets without clays. The study also saw an aflatoxin x clay interaction that indicated an increase in ADG and ADFI was greater for pigs fed contaminated diets than those fed control diets ($P < 0.10$) even though the control diets saw a higher gain:feed ratio ($P < 0.05$; Schell et al, 1993). When clay was added to aflatoxin contaminated diets fed to weanling and growing pigs, performance increased, whereas the gain:feed ratios decreased when pigs were fed strictly aflatoxin contaminated diets (Schell et al, 1993). Schell et al (1993) found that pigs fed contaminated feeds grew more slowly, consumed less feed and had a lower gain:feed ratio. When fed diets containing different types of clays, including sepiolite, calcium bentonite, and hydrated sodium calcium aluminosilicate (HSCA) there were higher average daily gains (ADG) and gain:feed ratios similar to those of pigs fed uncontaminated feeds. The 0.5% HSCA in a diet with 840ppb of mycotoxins saw the most prevention of reductions in daily gains and feed intake (Schell et al, 1993). In a research project by Lindemann et al (1997), pigs fed aflatoxin contaminated diets with

added clay saw feed intakes that were high enough to restore weight loss associated with the aflatoxins. At the end of the trial, there was a 27.8% reduction in growth rate associated with aflatoxins but a significant difference in feed intake was not observed. There were also significant behavioral changes that included wastage of contaminated feed because the pigs often rooted as though searching for better feed. There were four amendments made to the dietary treatments: a sodium bentonite clay was added to the control diet because of demonstrated ability to reduce aflatoxicosis, a kaolin clay was added to determine ability to prevent aflatoxicosis, and two mineral products: a pellet binder, as well as a Mg/Ca blend. Ultimately, the kaolin clay was determined to reverse negative effects of aflatoxin contaminated feed (Lindemann et al, 1997). The addition of bentonite clay with regards to aflatoxin and fumonisin B1 contamination in weaning diets will be addressed shortly.

The research has indicated that the use of feed additives are an important tool necessary to increase the biological utilization of nutrients in swine diets while reducing the negative aspects such as health and environmental effects. A further investigation into the use of enzymatically hydrolyzed yeast as a way to reduce nitrogen excretion would be useful in swine. A further investigation into the use of bentonite clay with regards to corn contaminated with more than just aflatoxin (e.g. fumonisin $B_{1)}$ in nursery diets would also be useful in swine.

Chapter 3. Effect of Supplementation of Enzymatically Hydrolyzed Yeast (EHY) on Digestibility in Finishing Pigs

3.1. Introduction

Feed additives are designed to improve many things including growth and feed efficiency. Antibiotic growth promoters (AGP) have been banned in the European Union and some are currently being voluntarily removed from the market in the United States, requiring other additives to be studied and potentially marketed as growth promoting. Yeast cultures have been used to improve nutrient digestibility of weaning pigs (Shen, 2009), as well as promoting feed intake (Bowman and Veum, 1973; Shen et al., 2009), increasing ADG and strengthening the immune system (Shen et al., 2009). It has been hypothesized that different strains of yeast may have differing properties and thus affect the microflora differently. The use of *S. cerevisiae* fermentation products has been shown to enhance intestinal morphology and thus allow pigs infected with *Salmonella* to have greater digestive capacity and show enhanced growth performance (Price, 2010).

The product used in this experiment was Celmanax®, an enzymatically hydrolyzed yeast (EHY) additive provided by Vi-Cor (Mason City, IA). It is a culture that contains hydrolyzed yeast, yeast extract and yeast culture of *Saccharomyces cerevisiae* (Vi-Cor, 2012). The following trial was conducted in order to test the effect of adding EHY to a finishing diet on performance and nutrient digestibility of dry matter, energy, and nitrogen. Another goal of the research was to determine if the length of time the pigs were on Celmanax was increased, if there would be an increase in performance and digestibility.

3.2. Materials and Methods

The experiment was conducted under protocols approved by the University of Kentucky's Institutional Animal Care and Use Committee.

3.2.1. Animals and Dietary Treatments

This experiment (experiment ID: UK1302) was carried out from January 2012 to March 2012 and utilized a total of 32 crossbred pigs [24 barrows; (Yorkshire x Landrace) x Duroc or Yorkshire x Duroc], with an initial body weight (BW) of 44.68 ± 4.28 kg. Pigs were brought into the University of Kentucky finishing facility and placed in an environmentally-controlled room at approximately 8 weeks of age and placed on a grow-finish diet adequate in all nutrients. The pigs were blocked by BW and litter and randomly allotted to one of four dietary treatments. The pigs were fed a complex grow-finish diet based on NRC (1998) nutrient requirements for pigs. The diets were arranged as a 2x2 factorial of lysine level (NRC vs reduced) and EHY (with and without) as follows: Diet 1 was a conventional corn-soybean meal diet, positive control (PC) diet meeting the SID lysine need (NRC, 1998) with no EHY; Diet 2 was the PC diet with 0.2% EHY; Diet 3 was a negative control diet (NC) with a 9% reduction in SID lysine with no EHY; Diet 4 was the NC diet with 0.2% EHY. Of the 32 initial pigs, the 24 most uniform pigs were used for the digestibility trial. Pigs were fed in the finishing room for 14 days and then divided into 2 groups of 12 pigs (3 replicates of each treatment per 12 pigs). The first group of 12 pigs was moved into the metabolism room and placed in stainless steel metabolic crates (49 x 37cm) for 14 days. Once they had been removed from the crates, the second group of 12 pigs was moved into the metabolism room for 14

days. When the second group had been removed from the metabolism room, the first group of 12 pigs was moved back into the metabolism crates. Pigs were provided with *ad libitum* access to water at all times. Feed allowance was restricted and based on 2.7% BW of each pig, of which they were fed half of the daily allowance in the morning and half of the daily allowance in the evening.

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

	Diet			
Ingredients	1	2	3	4
Corn	73.83	73.83	73.83	73.83
Soybean meal, 48% CP	22.00	22.00	19.00	19.00
Grease	1.00	1.00	1.00	1.00
Starch	0.50	0.30	3.50	3.30
L-lysine HCl	0.11	0.11	0.11	0.11
L-threonine	0.04	0.04	0.04	0.04
Dicalcium phosphate	0.85	0.85	0.85	0.85
Limestone	0.95	0.95	0.95	0.95
Salt	0.30	0.30	0.30	0.30
Vitamin Premix[a]	0.10	0.10	0.10	0.10
Mineral Premix[b]	0.08	0.08	0.08	0.08
AB-20 (clay)	0.25	0.25	0.25	0.25
Enzymatically Hydrolyzed Yeast	0.00	0.20	0.00	0.20
Total:	100.00	100.00	100.00	100.00
Calculated nutrient composition				
Crude protein, %	16.70	16.70	15.29	15.29
Lysine, %	0.83	0.83	0.75	0.75
Calcium, %	0.62	0.62	0.61	0.61
Phosphorus, %	0.52	0.52	0.50	0.50
Analyzed nutrient composition				
Dry matter, %	89.78	89.78	89.90	90.13
Gross energy, kcal/kg	3937	3937	3908	3908
Crude protein, %	16.99	16.84	16.08	15.42

[a] Supplied per kg of diet: 3300 IU of vitamin A, 660 IU of vitamin D, 33 IU of vitamin E, 3.3 mg of vitamin K, 4.4 mg of riboflavin, 11 mg of pantothenic acid, 44 mg of niacin, 16.5 ug of vitamin B_{12}, 110 ug of biotin, 660 ug of folic acid, and 3.3 mg of vitamin B_6

[b] Supplied per kg of diet: 8.25-10.75 mg of calcium, 8 mg of copper, 80 mg of iron, 30 mg of manganese, 100 mg of zinc, 1 mg of iodine, and 0.2 mg of selenium

3.2.2. Housing Conditions

A total of 8 finishing pens, with 4 pigs each, were used to house the pigs utilized in this trial. Of the 32 pigs, only 24 were ultimately chosen for the digestibility trial. The 24 pigs used for the digestibility trial were placed into two separate groups of 12 pigs and placed in 12 metabolism crates. The metabolism crates were made of stainless steel with plastic-coated expanded-metal flooring and metal feeders. Metabolism crates had a window in each side panel to allow visual contact between pigs in adjacent crates. In order to perform a total collection, a sliding aluminum screen and stainless steel funneled-pan were placed under the floor of the crates in order to collect and separate feces and urine. The interior space of the crates was set depending upon the size needs of the animal and also to stop the pigs from turning around in the crates.

3.2.3. Adaptation and Collection Methods

Pigs were housed in a finishing room for a period of 14 days in order to allow the GI tract to adapt to the diets. The pigs were weighed, blocked by weight and litter, and randomly allotted to the metabolism crates while remaining on whichever of the four diets to which they had been allotted. Once in the metabolism crates, the pigs were allowed 5 days of adaptation to the pens and restricted feeding. On the 5^{th} day, the pigs were weighed and no adjustments to feed were made. On the morning of the sixth day, 0.5% indigo carmine was added to the diet as a marker of the starting point of each collection period. Passage rate was the time it took for the indigo marker to show in the feces after being added to the feed. Pigs were provided *ad libitum* access to water with

feed allowance determined by 2.7%BW of each pig with half of the ration fed in the AM and half of the ration fed in the PM. Rejected feed was dried in a forced-air oven at 55C, air-equilibrated, weighed, and discounted from the amount initially offered. All of the feces produced during the period between excretion of the initial and final marker were collected daily and kept frozen in labeled plastic bags. All marked feces at the beginning of the collection period was included while all marked feces at the end of the period was excluded. Urine was collected for 5 days in 10L 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 remaining urine was discarded. The animals were placed into the metabolism pens in 2 groups of 12. The initial group of 12 pigs was placed into the crates a second time after the second group of 12 was taken out of the metabolism pens.

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

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

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

$$\text{Retention as a percent of intake, \%} = \left[\frac{Nutrients\ Retained}{Nutrient\ Intake}\right] x\ 100$$

Retention as a percent of absorption, % =

$$\left[\frac{Nutrients\ Retained}{Nutrient\ Intake - Nutrient\ Excretion\ (feces)}\right] x\ 100$$

3.2.4. Laboratory Analysis

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 4 days, then air-equilibrated, weighed, and ground through a 1mm screen using a Wiley Laboratory Mill (Model 3, Arthur H. Thomas Co., Philadelphia, PA). 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 record. Composite samples were kept frozen until analyzed.

Samples were analyzed in duplicate, and analysis was repeated when a coefficient of variation higher than 5% was observed in all samples analyzed with the exception of urinary nitrogen. For urinary N, samples with a variation of 10% or greater were re-analyzed. Dry matter in feed and feces was assessed according to an adaptation of the AOAC (1995) method, involving overnight drying (105°C) 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 using the equations previously described.

Gross energy content was assessed by bomb calorimetry, where samples are ignited in a pressurized-oxygen environment. The heat of combustion is considered the amount of energy transferred to a known mass of water contained in the calorimeter, using benzoic acid as the standard (Model 1261 Isoperibol Bomb Calorimeter, Parr Instruments Company, Moline, IL).

To measure urinary energy, samples were oven dried for 1 day at 100°C in polyethylene flat bags prior to combustion.

Urinary Energy=

$$\left[\frac{((E\ of\ bag\ and\ sample)x\ (bag\ and\ sample\ weight)) - ((bag\ wt)(E\ of\ bag))}{urine\ wt}\right]$$

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

3.2.5. Statistical Analysis

The experimental data was analyzed using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Each metabolism pen was considered an experimental unit for growth performance and digestibility measures. The statistical model included terms for collection, replicate, and treatment. The data was first run altogether to obtain collection,

treatment, and collection x treatment p-values and then run individually by collection period to obtain treatment and replicate p-values.

3.3. Results

A total of 3 collection periods were measured. As expected, there were differences between replicates within the collection periods, and between collection groups ($P<0.05$). There were no collection group x treatment interactions ($P>0.20$), therefore only main effect p-values are presented.

In this experiment as shown in Table 3.2, initial weight was affected by lysine content ($P = 0.05$) while final weight was not affected by lysine, EHY, or the interaction between the two. Average daily gain was affected by lysine ($P=0.07$) with the diets with a 9% reduction seeing decreased ADG. Apparent dry matter digestibility and feed intake were not affected by lysine, the EHY product, or an interaction between the two.

Energy digestibility, energy retention, digestible energy, and metabolizable energy ($P > 0.10$) were not affected by diet. Energy retention as a percent of absorbed was affected by lysine ($P=0.06$).

Nitrogen intake was affected by lysine content ($P < 0.0001$). Urinary nitrogen excretion was affected by the addition of the EHY product ($P=0.05$) as well as by the interaction between lysine and product ($P=0.09$). Fecal nitrogen excretion was not affected. Total nitrogen excretion was affected by both the EHY ($P=0.04$) and the interaction ($P=0.05$). Nitrogen absorption was affected by lysine content in the diet ($P<0.0001$). Nitrogen retention was affected by lysine ($P=0.08$) and the product ($P=0.05$).

Nitrogen digestibility was not affected by lysine, product, or interaction. Nitrogen retention as a percent of intake was affected by the product (P=0.04) as was retention as a percent of absorbed (P=0.05).

Table 3.2 Effect of EHY Supplementation on Digestibility

		Treatment[1]					Treatment P-values		
Response		1	2	3	4	PSEM	Lysine	EHY	Interaction
Performance									
	Initial Weight, kg	55.99	57.56	58.26	58.11	0.67	0.05	0.30	0.22
	Final Weight, kg	62.14	63.30	63.35	62.85	0.81	0.65	0.69	0.32
	ADG, kg/d	1.23	1.15	1.02	0.95	0.11	0.07	0.49	0.96
DM									
	Passage Rate (hours)	43.31	38.33	44.11	43.64	3.14	0.34	0.40	0.48
	Intake, kg/d	1.34	1.37	1.38	1.36	0.01	0.25	0.88	0.14
	Digestibility, %	90.51	89.40	90.03	90.13	0.40	0.77	0.22	0.15
Energy									
	Apparent digestibility, %	90.26	89.33	89.76	90.08	0.39	0.74	0.45	0.13
	Retention, % of intake	88.03	87.10	87.71	88.00	0.39	0.47	0.42	0.14
	Retention, % of absorbed	97.54	97.51	97.71	97.68	0.09	0.06	0.73	0.99
	Digestible Energy (kcal/kg of intake)	3553.21	3516.67	3507.83	3520.29	15.43	0.19	0.45	0.13
	Metabolizable Energy (kcal/kg of intake)	3465.65	3429.00	3427.51	3438.73	15.28	0.37	0.42	0.13
Nitrogen									
	Intake, g/d	40.39	41.15	38.81	38.21	0.43	<.0001	0.85	0.13
	Excreted in urine, g/d	12.66	15.65	13.51	13.72	0.77	0.49	0.05	0.09
	Excreted in feces, g/d	4.41	4.89	4.50	4.33	0.21	0.26	0.47	0.13
	Total excreted, g/d	17.07	20.54	18.01	18.04	0.80	0.34	0.04	0.05
	Absorption, g/d	35.97	36.25	34.31	33.89	0.39	<0.0001	0.86	0.38
	Retention, g/d	23.32	20.61	20.80	20.17	0.80	0.08	0.05	0.21
	Digestibility, %	89.07	88.07	88.39	88.74	0.48	0.99	0.50	0.17
	Retention, % of intake	57.62	50.34	54.35	52.66	2.05	0.82	0.04	0.19
	Retention, % of absorbed	64.71	57.21	61.54	59.27	2.35	0.82	0.05	0.28

[1] Values represent 12 pigs per treatment, with Diet 1 was a conventional corn-soybean meal, positive control (PC) diet meeting the SID lysine need (NRC, 1998) with no EHY; Diet 2 was a PC diet with 0.2% EHY; Diet 3 was a negative control diet (NC) with a 9% reduction in SID lysine with no EHY; Diet 4 was an NC diet with 0.2% EHY.

[2] PSEM-Pooled Standard Error of the Mean

Comparing Collection Means

As the pigs grew older and were on the diets for longer periods of time, we would expect to see increased absolute nitrogen retention due to the addition of the Celmanax®. These results were observed but there were inconsistencies in the progression, such as decreased urinary nitrogen excretion in collection period 2 yet largely increased excretion in collection period 3 (Table 3.3).

Table 3.3. Comparison of the Effect of EHY Supplementation on Digestibility Among Collection Periods

		Collection means[1]				
Response		1	2	3		PSEM
Performance						
	Initial Weight, kg	44.68	58.81	70.95		0.58
	Final Weight, kg	47.44	64.56	76.73		0.70
	ADG, kg/d	0.55	1.55	1.16		0.09
DM						
	Passage Rate (hours)	36.46	45.71	44.88		0.01
	Intake, kg/d	1.06	1.37	1.66		0.35
	Digestibility, %	90.03	89.38	90.64		2.72
Energy						
	Apparent digestibility, %	90.03	89.17	90.38		0.34
	Retention, % of intake	87.73	87.31	88.09		0.34
	Retention, % of absorbed	97.44	97.92	97.47		0.07
	Digestible Energy (kcal/kg of intake)	3532.40	3500.84	3540.26		13.36
	Metabolizable Energy (kcal/kg of intake)	3442.02	3428.01	3450.64		13.24
Nitrogen						
	Intake, g/d	30.62	39.54	48.76		0.37
	Excreted in urine, g/d	11.75	10.64	19.25		0.67
	Excreted in feces, g/d	3.53	4.60	5.47		0.18
	Total excreted, g/d	15.28	15.24	24.72		0.69
	Absorption, g/d	27.09	34.94	43.29		0.34
	Retention, g/d	15.33	24.30	24.04		0.69
	Digestibility, %	88.50	88.37	88.83		0.41
	Retention, % of intake	50.37	61.33	49.53		1.78
	Retention, % of absorbed	56.96	69.40	55.69		2.03

[1] Values represent 12 pigs per collection (4/trt); the pigs from collection 1 and 3 are the same at different time intervals

[2] PSEM-Pooled Standard Error of the Mean

Collection period 3 did not see increased digestibility from collection 2, in fact, it often saw similar or even lower numbers than collection 2. These collection means are means over all treatments. We would expect to see collection 1 and 2 to have similar numbers except DM digestibility (%) was decreased in collection 2 (89.38 compared to 1 at 90.03). Nitrogen intake (g/d) increased by collection 2 (30.62 in 1 and 39.54 in 2), yet urinary nitrogen excretion (g/d) decreased (11.75 to 10.64) but was up to 19.25 in collection period 3 (probably due to large N content that was unnecessary for older pigs).

Excretion in feces (g/d) increased by collection period. Total nitrogen excretion (g/d) was similar in collection 1 and 2 (15.28, 15.24) and large in collection 3 (24.72). Nitrogen absorption (g/d) increased as collection period increased (27.09, 34.94, 43.29) but collection 2 and 3 saw extremely similar nitrogen retention (g/d) at 24.30 and 24.04, much larger than collection 1's 15.3342. Nitrogen retention, % of intake saw 50.37, 61.33, 49.53 respectively and nitrogen retention, % of absorbed saw 56.96, 69.40, and 55.69. In both instances, the 3rd collection period saw less retention as a percentage basis than in the 1st and 2nd collections, with the 2nd collections showing the best numbers.

Comparing Collection 1 and Collection 3

The same pigs that were utilized in collection 1 were utilized in collection 3, the main difference being that by the time collection 3 was under way these pigs had been on the diets for 4 weeks. With regards to energy digestibility measures, the collection period 1 and collection period 3 data shows very little numerical differences. With regards to nitrogen, the intake of collection 3 was 48.76 g/d while collection 1 was 30.62g/d. Because of the larger nitrogen intake, there was also a larger nitrogen excretion of 19.25g/d excreted in the urine (compared to 11.75g/d in collection 1) and 5.47g/d excreted in the feces (compared to 3.53g/d), coming to a total of 24.72g/d of nitrogen excreted which was much larger than the 15.28g/d of nitrogen excreted in collection 1. Absorption and retention in terms of g/d were greater in collection 3 but in terms of % of intake and % absorbed, collection 3 saw a smaller performance than in collection 1.

3.4. Discussion

Yeast products have been touted as a feed additive that will improve growth, feed efficiency, and reduce nutrient excretion. The observations from this research agree with that of Bowman and Veum (1973) in finding that *S. cerevisiae* yeast added to finishing diets does not affect growth performance. Unlike Shen et al (2009) which concluded that yeast cultures promote feed intake and increase ADG, the current results do not support the promotion of feed intake and increased ADG of pigs fed yeast cultures. In fact, the diet that saw the greatest ADG, least feed intake, lowest nitrogen excretion and greatest nitrogen retention was the control diet which met the lysine need and did not have added yeast.

3.5. Conclusions

The purpose of this experiment was to determine whether enzymatically hydrolyzed yeast (EHY) added to swine diets would change the digestibility, as well as nitrogen excretion, of that diet. The data shows that the diets containing EHY saw a greater nitrogen excretion rate than the conventional diets. It is possible that the pigs were unable to utilize the excess nitrogen to their advantage and were forced to excrete it. Overall there was little proof to support an increase in performance with the addition of EHY to the finishing swine diets. It is possible that the inclusion value of the product was too low to see a significant change in performance values. Another cause for concern was the variability in the pigs as they grew which may have superceded any noticeable differences in dietary effects. Last but not least we may have seen a dramatic increase in nitrogen intake and excretion in the pigs from collection 3 because they were on a

growing diet much longer when a finishing diet would have been more appropriate by the time they reached the third collection, but in the interest of simplicity, we utilized only one diet phase during the experiment. Further research utilizing yeast products in weaning pigs as well as use when feeding alternative feeds would be helpful. As far as use of yeasts in finishing swine diets, it does not seem to be an important tool for growth performance.

Chapter 4. Effect of feeding naturally-contaminated corn on nutrient digestibility and feed preference in weanling pigs

4.1. Introduction

Mycotoxins in animal feed are a concern due to the physiological effects from ingestion of mycotoxin contaminated feed as well as the associated economic losses. Swine and poultry are particularly susceptible to mycotoxins and focus in research has been to find ways to mitigate the damage with feed additives designed to bind to mycotoxins to prevent absorption or to metabolize them and thereby to reduce mycotoxicoses. Mycotoxins are linked to birth defects, nervous system issues, tumors, and many other issues. Aflatoxins are the most widely recognized mycotoxin because they are considered carcinogenic and attack the liver and immune system, which makes them a severe health risk to animals and humans. Clays are the simplest additive used to prevent adverse effects associated with mycotoxin contaminated grains. The disadvantages of feeding contaminated feed to livestock includes reduced feed intake, lowered daily gains, and reduced feed efficiency (Harvey et al, 1988, Lindemann et al, 1988). Pigs fed aflatoxin-contaminated diets containing clay consumed more and grew faster ($P < 0.01$) than pigs fed contaminated diets without clays. The study also saw an aflatoxin x clay interaction that indicated an increase in ADG and ADFI was greater for pigs fed contaminated diets than those fed control diets ($P < 0.10$) even though the control diets saw a higher gain:feed ratio ($P < 0.05$; Schell et al, 1993). In a research trial by Lindemann et al (1997), at the end of the trial, there was a 27.8% reduction in growth rate associated with aflatoxins but a significant difference in feed intake was not observed. Pigs fed aflatoxin contaminated diets with added kaolin clay saw feed intakes that were

high enough to restore weight loss associated with the aflatoxins. There were also significant behavioral changes that included wastage of contaminated feed because the pigs often rooted as though searching for better feed. There were four amendments made to the dietary treatments: a sodium bentonite clay was added to the control diet because of demonstrated ability to reduce aflatoxicosis, a kaolin clay was added to determine ability to prevent aflatoxicosis, and two mineral products: a pellet binder, as well as a Mg/Ca blend. Ultimately, the kaolin clay was determined to reverse negative effects of aflatoxin contaminated feed (Lindemann et al, 1997). The current research will investigate the possible benefit of using a sodium bentonite clay as a binder for corn contaminated with aflatoxins and fumonisin B_1.

4.2. 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 for use in 2 experiments.

4.2.1. Animals and Dietary Treatments

Dietary Treatments: Diet 1 was a conventional corn-soybean meal, positive control (PC) diet made with 2011 corn; Diet 2 was a negative control (NC) diet made with naturally-contaminated 2012 corn; Diet 3 was the PC diet with sodium bentonite clay (AB-20,

Prince Agri Products, Inc., Quincy, Il); Diet 4 was the NC diet with sodium bentonite clay (see Tables 4.1 and 4.2).

Experiment 1: This experiment (experiment ID: UK1309) was carried out during October and November 2013 and utilized a total of 36 crossbred pigs [18 barrows, 18 gilts; (Yorkshire x Landrace) x Duroc or Yorkshire x Duroc], with an initial body weight (BW) of 10.49 ± 1.09 kg. This experiment was designed to determine if pigs would exhibit a preference for diets based on corn quality and/or dietary amendment. The pigs were blocked by BW and litter and allotted to three dietary comparisons, Control vs Diet 2 (Comparison 1), Control vs Diet 4 (Comparison 2), and Diet 2 vs Diet 4 (Comparison 3). Each comparison involved 3 pens, each with 4 pigs (two barrows and two gilts). Pigs were fed a common nursery diet for 7 days and then allotted to comparisons. Pigs were provided with *ad libitum* access to feed and water for each of the two 1-week periods. There were two feeders placed in each pen in order to determine feed preference. The feeder positions were changed three times a week.

Experiment 2: This experiment (experiment ID: UK1310) was carried out during October and November 2013 and utilized a total of 24 crossbred pigs [12 barrows, 12 gilts; (Yorkshire x Landrace) x Duroc or Yorkshire x Duroc], with an initial body weight (BW) of 8.09 ± 1.13 kg. The pigs were blocked by BW and litter and randomly allotted to one of the four dietary treatments. The piglets were fed a common diet in the nursery for 7 days then moved into the metabolism room and placed in stainless steel metabolic pens

(49 x 37cm), with each pen containing 2 pigs (1 barrow and 1 gilt) for 19 days. Pigs were provided with *ad libitum* access to feed and water at all times.

Table 4.1 Composition of Experimental Diets for Nursery Pigs (%, as-fed basis)				
	Diet			
Ingredients	1	2	3	4
Ground corn (2011, non contaminated)	57.10	17.13	57.10	17.13
Ground corn (2012, contaminated)		39.97		39.97
Soybean meal, 48% CP	19.35	19.35	19.35	19.35
Fish meal, menhaden	4.00	4.00	4.00	4.00
Spray dried animal plasma	2.50	2.50	2.50	2.50
Dried whey	12.50	12.50	12.50	12.50
Choice white grease	1.95	1.95	1.95	1.95
Dicalcium phosphate	0.90	0.90	0.90	0.90
Limestone	0.60	0.60	0.60	0.60
Salt	0.21	0.21	0.21	0.21
Vitamin/TM premix[a]	0.15	0.15	0.15	0.15
Choline chloride, 60%	0.05	0.05	0.05	0.05
Santoquin	0.02	0.02	0.02	0.02
Mecadox-10[b]	0.25	0.25	0.25	0.25
L-lysine, HCL	0.19	0.19	0.19	0.19
L-threonine	0.09	0.09	0.09	0.09
DL-methionine	0.14	0.14	0.14	0.14
Sodium Bentonite Clay, %			0.50	0.50
Starch, %	0.50	0.50		
Total:	100.50	100.50	100.50	100.50
Calculated nutrient composition				
ME, kcal/kg	3394.00	3394.00	3394.00	3394.00
Crude protein, %	20.24	20.24	20.24	20.24
Lysine, %	1.23	1.23	1.23	1.23
Calcium, %	0.80	0.80	0.80	0.80
Phosphorus, %	0.70	0.70	0.70	0.70
Available phosphorus, %	0.45	0.45	0.45	0.45
Analyzed nutrient composition				
Dry matter, %	90.72	90.35	90.52	91.09
Gross energy, kcal/kg	3914.32	3899.20	3899.88	3856.25
Crude protein, %	22.21	22.47	21.53	20.21

[a] Supplied per kg of feed: 0.3 mg of Selenium, 10.95 mg of Zinc, 9007 IU of Vitamin A, 2253 IU of Vitamin D3, 60 IU of Vitamin E (Sow A VTM Premix SE-Yeast; Provimi North America, Brookville, Ohio)

[b] Mecadox 10 (Medicated Article supplying Carbadox; Pfizer Inc., Exton, PA) was added at 55 mg/kg of diet.

Table 4.2 Mycotoxin Concentration in 2011 and 2012 Corn					
Mycotoxin	2011 corn, ppm[a]	2012 corn, ppm[b]	PC diet with 100% of 2011 corn, ppm	NC diet with 70% of 2012 corn, ppm	Critical levels for growing pigs, ppm[c]
Aflatoxin B1	<0.02	0.07	ND	0.03	<0.1
DON	<0.5	<0.5	ND	ND	<1
15-Acetyl DON	<0.5	<0.5	ND	ND	No reports
Fumonisin B1[d]	<2.0	73.0	ND	29.20	<10
Zearalenone	<0.5	<0.5	ND	ND	<1[4]
[a] Corn was analyzed by the Veterinary Diagnostic Laboratory at North Dakota State University					
[b] Corn was analyzed by the Veterinary Diagnostic Laboratory at North Dakota State University					
[c] 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.					
[d]Fumonisin B1 concentration reported at 57ppm by University of Missouri and 10.3ppm by Iowa State University					

4.2.2. Housing Conditions

Experiment 1: A total of 9 nursery pens, with 4 pigs each, were used to conduct this preference trial. Pigs were housed in elevated nursery pens with plastic coated, welded wire flooring (1.22 m x 1.22 m). Each pen had a nipple waterer and 2 plastic nursery feeders.

Experiment 2: A total of 12 metabolism pens, with 2 pigs each, were used to conduct a balance trial. The metabolism pens were made of stainless steel with plastic-coated expanded-metal flooring and plastic feeders. Each pen had a nipple waterer. Metabolism pens had a window in each side panel to allow visual contact between pigs in adjacent pens. In order to perform a total collection, a sliding aluminum screen and stainless steel funneled-pan were placed under the floor of the pens in order to separate and collect feces and urine.

4.2.3. Adaptation and Collection Methods

Experiment 1: Pigs were housed in the nursery for 7 days and fed a common diet before being placed on trial. The pigs were weighed, blocked by sex and weight, and allotted to preference pens (three possible comparisons). The experiment included two periods of 1 week. At the beginning of each week all animals and feeders were weighed. The feeders in the pens were moved 3 times a week to reduce the possibility that pigs were feeding from a preferred space and not from a preferred diet.

Experiment 2: Pigs were housed in the nursery for 7 days and fed a common diet before being placed on trial. The pigs were weighed, blocked by sex and weight, and randomly allotted to the metabolism pens. There were three collection periods, the first two lasting seven days and the final period lasted five days. Pigs and feeders were weighed at the beginning and end of each period and 0.5% indigo carmine was added to the diet as a visual marker of the starting point of each collection period. Passage rate was the time it took for the indigo marker to show in the feces after being added to the feed. Pigs were provided *ad libitum* access to feed and water. All marked feces at the beginning of the collection period was included while all marked feces at the end of the period was excluded. Urine was collected for 7 days for the first 2 periods and for 5 days in the 3rd and final period in 10L 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 remaining urine was discarded.

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

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

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

$$\text{Retention as a percent of intake, \%} = \left[\frac{Apparent\ Nutrients\ Retained}{Nutrient\ Intake}\right] x\ 100$$

Retention as a percent of absorption, % =

$$\left[\frac{Apparent\ Nutrients\ Retained}{Nutrient\ Intake - Nutrient\ Excretion\ (feces)}\right] x\ 100$$

4.2.4. Laboratory Analysis

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 necessary.

All frozen feces were dried in a forced-air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) at 55°C for 4 days, then air-equilibrated, weighed, and ground through a 1mm screen using a Wiley Laboratory Mill (Model 3, Arthur H. Thomas Co.,

Philadelphia, PA). 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 record. Composite samples were kept frozen until analyzed.

Samples were analyzed in duplicate, and analysis was repeated when a coefficient of variation higher than 5% was observed in all samples analyzed with the exception of urinary nitrogen. Samples with a variation of 10% or greater were re-analyzed with regards to urinary nitrogen. Dry matter in feed and feces was assessed according to an adaptation of the AOAC (1995) method, involving overnight drying (105°C) 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 using the equations previously described.

Gross energy content was assessed by bomb calorimetry, where samples are ignited in a pressurized-oxygen environment. The heat of combustion is considered the amount of energy transferred to a known mass of water contained in the calorimeter, using benzoic acid as the standard (Model 1261 Isoperibol Bomb Calorimeter, Parr Instruments Company, Moline, IL).

To measure urinary energy, samples were oven dried for 1 day at 100°C in polyethylene bags prior to combustion.

Urinary Energy=

$$\left[\frac{((E\ of\ bag\ and\ sample)x\ (bag\ and\ sample\ weight)) - ((bag\ wt)(E\ of\ bag))}{urine\ wt}\right]$$

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

4.2.5. Statistical Analysis

Experiment 1: The data was analyzed by unpaired T-tests using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). The data was considered significant at alpha=0.05.

Experiment 2: The experimental data was analyzed using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Each metabolism pen was considered an experimental unit for growth performance and digestibility measures. The statistical model included terms for collection, replicate, and treatment. The entire data set was first run to obtain collection, treatment, and collection x treatment p-values and then run individually by collection period to obtain treatment p-values within each collection.

4.3. Results

4.3.1. Experiment 1

A preference was shown for the normal corn (Diet 1) over the mycotoxin contaminated corn (Diet 2) and added clay (Diet 4) in Table 4.3 and Table 4.4. The preference was most clearly shown in the comparison of Diet 1 versus Diet 4 with both Week 1 and Week 2, as well as the entire period (2 weeks total), having p-values of

<0.0001. The preference for Diet 1 was obvious (Week 1: 91.34% vs. 8.66%; Week 2: 93.77% vs. 6.23%; Overall: 92.72% vs. 7.28%). The comparison between Diet 1 and Diet 2 shows a numerical advantage towards preferring the control diet in the first period (70.7% vs. 29.3%) but a statistically significant preference towards the control diet during the second period (92.16% vs. 7.84%; P<0.0001). Comparison 3 (Table 4.5) between Diet 2 and Diet 4 showed a preference towards the naturally-contaminated corn (Diet 2) instead of the naturally-contaminated corn with added clay (Diet 4) (Week 1: 79.35% vs. 20.65%; Week 2: 72.73% vs. 27.27%; Overall: 75.57% vs. 24.43%; P<0.05) even though the preference was not as pronounced as the preference for the traditional corn-soybean meal diet instead of the naturally-contaminated corn diets.

Table 4.3 Effect of Naturally Contaminated Corn on Feed Preference

	Body Weight		Comparison 1 (Control vs Diet 2)		
Period	Initial wt, kg	Final wt, kg	Control	Diet 2	P-value
1	10.46	15.02	70.70	29.30	0.1220
2	15.02	20.69	92.16	7.84	< 0.0001
1 & 2	10.46	20.69	84.06	15.94	0.0003

[1] Values represent 3 pens with 4 pigs per pen, with Control (Diet 1) a conventional corn-soybean meal, positive control (PC) diet; Diet 2 a NC diet.

Table 4.4 Effect of Naturally Contaminated Corn on Feed Preference

	Body Weight		Comparison 2 (Control vs Diet 4)		
Period	Initial wt, kg	Final wt, kg	Control	Diet 4	P-value
1	10.50	15.26	91.34	8.66	< 0.0001
2	15.26	19.68	93.77	6.23	< 0.0001
1 & 2	10.50	19.68	92.72	7.28	< 0.0001

[1] Values represent 3 pens with 4 pigs per pen, with Control (Diet 1) a conventional corn-soybean meal, positive control (PC) diet; Diet 4 a NC diet with clay.

Table 4.5 Effect of Naturally Contaminated Corn on Feed Preference					
	Body Weight		Comparison 3 (Diet 2 vs Diet 4)		
Period	Initial wt, kg	Final wt, kg	Diet 2	Diet 4	P-value
1	10.51	15.30	79.35	20.65	0.0289
2	15.30	19.73	72.73	27.27	0.0463
1 & 2	10.51	19.73	75.57	24.43	0.0375

[1] Values represent 3 pens with 4 pigs per pen, with Diet 2 a NC diet; Diet 4 a NC diet with clay.

There were no significant differences in body weight, daily gain, daily feed intake, or the gain to feed ratio among the three different comparisons (Table 4.6). It should be noted that Comparison 3 did not have a PC diet as an option for consumption.

Table 4.6 Effect of Naturally Contaminated Corn on Perfomance in Nursery Pigs						
	Comparisons					P-value
Performance trait	1	2	3	SE		
Body weight, kg						
Initial	10.46	10.50	10.51	0.11		0.84
Final	19.78	19.68	19.74	0.93		0.99
Daily gain, kg						
Phase I	0.65	0.68	0.69	0.08		0.77
Phase II	0.68	0.63	0.63	0.08		0.63
Phase I & II	0.67	0.66	0.66	0.07		0.97
Daily feed, kg						
Phase I	0.81	0.85	0.86	0.14		0.81
Phase II	1.15	1.07	1.16	0.08		0.28
Phase I & II	0.98	0.96	1.01	0.09		0.69
Gain:feed						
Phase I	0.82	0.81	0.80	0.07		0.98
Phase II	0.59	0.59	0.55	0.02		0.42
Phase I & II	0.68	0.69	0.65	0.04		0.80

[1] Comparison 1 was a comparison between Diet 1 (PC) and Diet 2 (NC)

[2] Comparison 2 was a comparison between Diet 1 (PC) and Diet 4 (NC with clay)

[3] Comparison 3 was a comparison between Diet 2 (NC) and Diet 4 (NC with clay)

[4] Phase I was Week 1 of the trial; Phase II was Week 2 of the trial (both were 7 days)

4.3.2. Experiment 2

In the first collection period as shown in Table 4.7, final weight was affected by the interaction of clay and corn quality (P=0.04) with the pigs on the non-contaminated corn with starch and pigs on the contaminated corn with clay having similar weights while the pigs on corn with clay and pigs on contaminated corn had similar weights. Average daily gain (ADG) was also affected by the interaction between corn quality and clay (P=0.03).

Apparent dry matter digestibility and feed intake were not affected by corn quality, clay, or an interaction between the two.

Energy digestibility, energy retention, digestible energy, and metabolizable energy ($P > 0.10$) were not affected by diet.

Nitrogen intake was decreased by corn quality ($P < 0.05$) with the contaminated corn diets resulting in less nitrogen intake per pig. Nitrogen excretion was not affected by corn quality, clay, or interaction between the two. Nitrogen absorption and retention was affected by corn quality in the diet ($P<0.05$). Nitrogen digestibility was not affected by corn quality, clay, or interaction. Nitrogen retention as a percent of intake was decreased by corn quality (P=0.07) as was retention as a percent of absorbed (P=0.05).

Table 4.7. Effect of Naturally Contaminated Corn on Digestibility During Collection Period 1									
		Treatment[1]					P-value		
Response		1	2	3	4	PSEM[2]	Corn Quality	Clay	Interaction
Performance									
	Initial Weight, kg	8.11	8.13	8.02	8.11	0.07	0.50	0.47	0.67
	Final Weight, kg	10.93	11.39	11.30	10.74	0.20	0.82	0.49	0.04
	ADG, kg/d	0.40	0.47	0.47	0.38	0.03	0.63	0.66	0.03
DM									
	Passage Rate (hours)	16.08	15.92	11.50	15.50	3.14	0.56	0.46	0.53
	Intake, kg/d	0.86	0.73	0.86	0.72	0.03	0.01	0.80	0.76
	Digestibility, %	93.93	92.39	94.10	93.41	0.83	0.23	0.50	0.63
Energy									
	Apparent digestibility, %	93.72	92.24	94.01	94.00	1.11	0.53	0.39	0.53
	Retention, % of intake	91.33	90.00	91.39	91.37	1.29	0.62	0.60	0.63
	Retention, % of absorbed	97.45	95.58	97.21	97.21	0.28	0.82	0.31	0.82
	Digestible Energy (kcal/kg of intake)	4179.05	4047.48	4082.39	3438.58	294.67	0.24	0.28	0.42
	Metabolizable Energy (kcal/kg of intake)	3568.15	3516.22	3544.06	3543.55	49.95	0.62	0.98	0.63
Nitrogen									
	Intake, g/d	33.20	27.49	33.25	26.79	1.18	< 0.05	0.79	0.76
	Excreted in urine, g/d	3.58	3.22	3.08	3.26	0.30	0.79	0.47	0.41
	Excreted in feces, g/d	2.60	2.71	2.48	2.19	0.28	0.77	0.29	0.49
	Total excreted, g/d	6.17	5.94	5.55	5.45	0.44	0.71	0.25	0.89
	Absorption, g/d	30.60	24.77	30.78	24.61	1.29	<0.05	1.00	0.90
	Retention, g/d	27.03	21.55	27.71	21.35	1.16	<0.05	0.85	0.72
	Digestibility, %	92.23	90.05	92.58	91.48	1.22	0.23	0.49	0.67
	Retention, % of intake	81.57	78.63	83.32	79.34	1.60	0.07	0.47	0.76
	Retention, % of absorbed	88.41	87.32	90.00	86.74	0.91	0.05	0.60	0.27

[1] Values represent 3 pens with 2 pigs/pen except on kg/d and g/d measurements (1 pig) Diets were complex nursery diets as follows: Diet 1 was a conventional corn-soybean meal, positive control (PC) diet; Diet 2 contained naturally contaminated corn (NC diet); Diet 3 was a PC diet with sodium bentonite clay; Diet 4 was a NC diet with sodium bentonite clay.

[2] PSEM-Pooled Standard Error of the Mean

[3] Collection Period was 7 days.

In the second collection period as shown in Table 4.8, final weight was affected by the different diets (P=0.09) with the pigs on the contaminated corn with clay having the lowest weights. Average daily gain (ADG) was not affected by diet (P>0.10).

Passage rate was affected by corn quality (P=0.07) with the naturally-contaminated corn diets seeing an increased time required for passage. Apparent dry matter digestibility was not affected corn quality, clay or interaction. Feed intake was affected by corn quality (P=0.10) with the contaminated corn diets having a lower feed intake.

Energy digestibility, energy retention, digestible energy, and metabolizable energy (P > 0.10) were not affected by diet.

Nitrogen intake was affected by corn quality (P = 0.05). Nitrogen excretion was not affected by corn quality, clay or the interaction between the two. Nitrogen absorption was affected by corn quality (P=0.04) as was nitrogen retention (P=0.02) with a greater absorption and retention (g/d) in the PC diets. Nitrogen digestibility was affected by corn quality (P=0.05) with the PC diets having a greater digestibility. Nitrogen retention as a percent of intake was affected by the corn quality (P=0.02) as was retention as a percent of absorbed (P=0.05).

Table 4.8. Effect of Naturally Contaminated Corn on Digestibility During Collection Period 2

		Treatment[1]					P-value		
Response		1	2	3	4	PSEM[2]	Corn Quality	Clay	Interaction
Performance									
	Initial Weight, kg	10.93	11.39	11.30	10.74	0.20	0.82	0.49	0.04
	Final Weight, kg	15.37	15.31	15.59	14.36	0.32	0.09	0.30	0.12
	ADG, kg/d	0.63	0.56	0.61	0.52	0.05	0.13	0.55	0.84
DM									
	Passage Rate (hours)	23.33	27.33	20.17	27.33	2.52	0.07	0.55	0.55
	Intake, kg/d	0.81	0.72	0.79	0.67	0.05	0.10	0.53	0.79
	Digestibility, %	89.22	88.41	89.14	87.68	0.70	0.16	0.58	0.65
Energy									
	Apparent digestibility, %	89.28	88.32	89.13	87.97	0.77	0.22	0.75	0.90
	Retention, % of intake	86.30	85.08	85.22	84.15	0.97	0.28	0.34	0.94
	Retention, % of absorbed	96.65	96.33	95.61	95.66	0.47	0.79	0.12	0.71
	Digestible Energy (kcal/kg of intake)	3827.71	3831.66	3815.96	3667.94	52.13	0.22	0.14	0.20
	Metabolizable Energy (kcal/kg of intake)	3371.44	3324.01	3304.79	3263.32	37.82	0.28	0.14	0.94
Nitrogen									
	Intake, g/d	31.10	26.98	30.33	25.16	1.93	0.05	0.53	0.80
	Excreted in urine, g/d	4.83	5.38	4.80	4.86	0.54	0.59	0.63	0.66
	Excreted in feces, g/d	3.87	4.02	3.84	3.69	0.30	0.99	0.56	0.63
	Total excreted, g/d	8.70	9.40	8.64	8.55	0.73	0.69	0.55	0.61
	Absorption, g/d	27.23	22.96	26.49	21.47	1.75	0.04	0.55	0.84
	Retention, g/d	22.40	17.58	21.69	16.62	1.54	0.02	0.61	0.94
	Digestibility, %	87.54	85.10	87.34	85.21	0.96	0.05	0.96	0.87
	Retention, % of intake	72.20	66.05	71.42	65.95	1.92	0.02	0.83	0.86
	Retention, % of absorbed	82.44	77.61	81.75	77.45	1.82	0.05	0.82	0.89

[1] Values represent 3 pens with 2 pigs/pen except on kg/d and g/d measurements (1 pig) Diets were complex nursery diets as follows: Diet 1 was a conventional corn-soybean meal, positive control (PC) diet; Diet 2 contained naturally contaminated corn (NC diet); Diet 3 was a PC diet with sodium bentonite clay; Diet 4 was a NC diet with sodium bentonite clay.

[2] PSEM-Pooled Standard Error of the Mean

3 Collection Period was 7 days.

In the third collection period as shown in Table 4.9, weight and ADG were not affected by corn quality, clay, or the interaction.

Passage rate was affected by corn quality (P=0.01) with contaminated corn diets seeing slower passage. Apparent dry matter digestibility and feed intake were not affected by corn quality, clay, or an interaction between the two.

Energy digestibility, energy retention, and metabolizable energy (P > 0.10) were not affected by diet. Digestible energy was affected by corn quality (P=0.04) with the PC diets having larger kcal/kg of intakes.

Nitrogen intake and nitrogen excretion were not affected by corn quality, clay or the the interaction (P>0.10). Nitrogen absorption was affected by corn quality (P=0.01) as was nitrogen retention (P=0.04) with the control diet having the most retention (28.48 g/d). Nitrogen digestibility was affected by corn quality (P=0.09) with the PC diets seeing 80.96 and 80.24% digestibility compared to 75.11 and 76.10% digestibility in the naturally-contaminated corn. Nitrogen retention as a percent of intake and nitrogen retention as a percent of absorbed were not affected (P>0.10).

Table 4.9. Effect of Naturally Contaminated Corn on Digestibility During Collection Period 3

		Treatment[1]					P-value		
Response		1	2	3	4	PSEM[2]	Corn Quality	Clay	Interaction
Performance									
	Initial Weight, kg	15.37	13.03	15.59	14.36	1.19	0.18	0.54	0.66
	Final Weight, kg	18.17	18.76	18.50	18.14	0.36	0.76	0.70	0.24
	ADG, kg/d	0.56	1.15	0.58	0.76	0.23	0.16	0.46	0.42
DM									
	Passage Rate (hours)	28.17	35.33	30.83	35.67	1.74	0.01	0.42	0.53
	Intake, kg/d	1.12	1.12	1.06	1.06	0.04	1.00	0.13	1.00
	Digestibility, %	83.52	80.55	83.37	81.09	1.65	0.16	0.91	0.84
Energy									
	Apparent digestibility, %	83.17	79.93	82.91	81.15	1.79	0.21	0.80	0.70
	Retention, % of intake	80.09	76.87	79.46	78.27	1.85	0.28	0.84	0.60
	Retention, % of absorbed	96.29	96.14	95.83	96.45	0.25	0.37	0.76	0.17
	Digestible Energy (kcal/kg of intake)	3661.36	3535.71	3654.65	3547.03	62.25	0.04	0.52	0.58
	Metabolizable Energy (kcal/kg of intake)	3128.94	3003.00	3081.44	3035.23	71.95	0.28	0.92	0.60
Nitrogen									
	Intake, g/d	43.38	42.08	40.74	39.51	1.50	0.43	0.13	0.98
	Excreted in urine, g/d	6.60	6.68	7.03	6.28	0.64	0.62	0.98	0.54
	Excreted in feces, g/d	8.31	10.54	8.11	9.48	1.34	0.23	0.66	0.76
	Total excreted, g/d	14.91	17.22	15.14	15.76	1.69	0.42	0.73	0.63
	Absorption, g/d	35.07	31.53	32.63	30.03	0.72	0.01	0.03	0.54
	Retention, g/d	28.48	24.85	25.60	23.75	1.06	0.04	0.11	0.43
	Digestibility, %	80.96	75.11	80.24	76.10	2.45	0.09	0.96	0.74
	Retention, % of intake	66.06	59.18	62.89	60.25	2.97	0.16	0.74	0.50
	Retention, % of absorbed	81.52	78.43	78.36	79.20	2.09	0.61	0.59	0.38

[1] Values represent 3 pens with 2 pigs/pen except on kg/d and g/d measurements (1 pig) Diets were complex nursery diets as follows: Diet 1 was a conventional corn-soybean meal, positive control (PC) diet; Diet 2 contained naturally contaminated corn (NC diet); Diet 3 was a PC diet with sodium bentonite clay; Diet 4 was a NC diet with sodium bentonite clay.

[2] PSEM-Pooled Standard Error of the Mean

3 Collection Period was 5 days.

With each collection period, DM, energy and nitrogen digestibility decreased with regards to each diet (see Figures 4.1, 4.2, 4.3). In this experiment as shown in Table 4.10, final weight was affected by the interaction between corn quality and clay (P=0.01). Average daily gain (ADG) was not affected by diet (P>0.10).

Table 4.10. Effect of Naturally Contaminated Corn on Digestibility

		Treatment[1]					P-value		
Response		1	2	3	4	PSEM[2]	Corn Quality	Clay	Interaction
Performance									
	Initial Weight, kg	11.47	10.85	11.64	11.07	0.40	0.16	0.64	0.95
	Final Weight, kg	14.82	15.15	15.13	14.41	0.17	0.28	0.22	0.01
	ADG, kg/d	0.53	0.72	0.55	0.55	0.08	0.26	0.36	0.24
DM									
	Passage Rate (hours)	22.53	26.19	20.83	26.17	1.46	0.01	0.56	0.58
	Intake, kg/d	0.93	0.86	0.90	0.81	0.02	<0.05	0.14	0.74
	Digestibility, %	88.89	87.12	88.87	87.39	0.66	0.02	0.85	0.83
Energy									
	Apparent digestibility, %	88.72	86.83	88.68	87.71	0.75	0.07	0.59	0.55
	Retention, % of intake	85.91	83.98	85.35	84.60	0.82	0.12	0.97	0.49
	Retention, % of absorbed	96.79	96.69	96.21	96.44	0.20	0.77	0.05	0.41
	Digestible Energy (kcal/kg of intake)	3889.37	3804.95	3851.00	3521.18	101.88	0.06	0.13	0.24
	Metabolizable Energy (kcal/kg of intake)	3356.17	3281.08	3310.10	3280.70	31.80	0.12	0.47	0.48
Nitrogen									
	Intake, g/d	35.90	32.18	34.77	30.49	0.91	<0.05	0.14	0.76
	Excreted in urine, g/d	5.00	5.09	4.97	4.80	0.30	0.90	0.59	0.66
	Excreted in feces, g/d	4.93	5.76	4.81	5.12	0.47	0.24	0.43	0.58
	Total excreted, g/d	9.93	10.85	9.78	9.92	0.63	0.41	0.40	0.54
	Absorption, g/d	30.97	26.42	29.97	25.37	0.76	<.0001	0.20	0.97
	Retention, g/d	25.97	21.33	25.00	20.57	0.73	<.0001	0.25	0.88
	Digestibility, %	86.91	83.42	86.72	84.26	0.97	0.01	0.74	0.60
	Retention, % of intake	73.28	67.95	72.54	68.52	1.29	<0.05	0.95	0.62
	Retention, % of absorbed	84.12	81.12	83.37	81.13	0.97	0.01	0.71	0.70

[1] Values represent 3 pens with 2 pigs/pen except on kg/d and g/d measurements (1 pig) Diets were complex nursery diets as follows: Diet 1 was a conventional corn-soybean meal, positive control (PC) diet; Diet 2 contained naturally contaminated corn (NC diet); Diet 3 was a PC diet with sodium bentonite clay; Diet 4 was a NC diet with sodium bentonite clay.

[2] PSEM-Pooled Standard Error of the Mean

[3] Total Collection Period was 19 days-Period 1 & 2 days were 7 days long each, Period 3 was 5 days long.

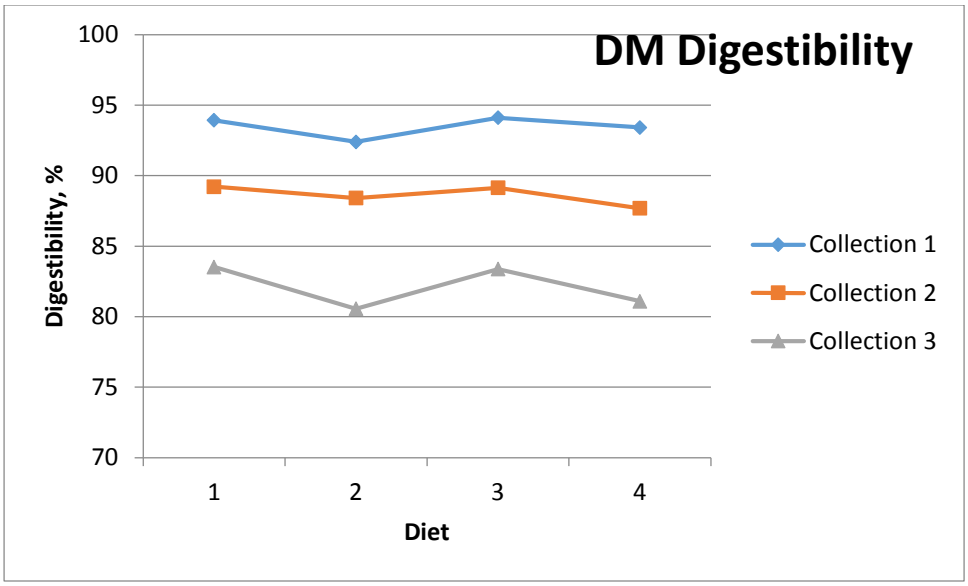


Figure 4.1 Effect of Naturally-Contaminated Corn on DM Digestibility. Each mean represents 4 pens/treatment with 2 pigs/pen. The experimental period for collection 1 and 2 was 7 days, collection 3 was 5 days. Diets were complex nursery diets as follows: Diet 1 was a conventional corn-soybean meal, positive control (PC) diet; Diet 2 contained naturally-contaminated corn (NC diet); Diet 3 was a PC diet with sodium bentonite clay; Diet 4 was a NC diet with sodium bentonite clay. Corn quality ($P < 0.05$) and collection ($P<0.0001$) effects were observed; no collection x treatment effect ($P=0.93$).

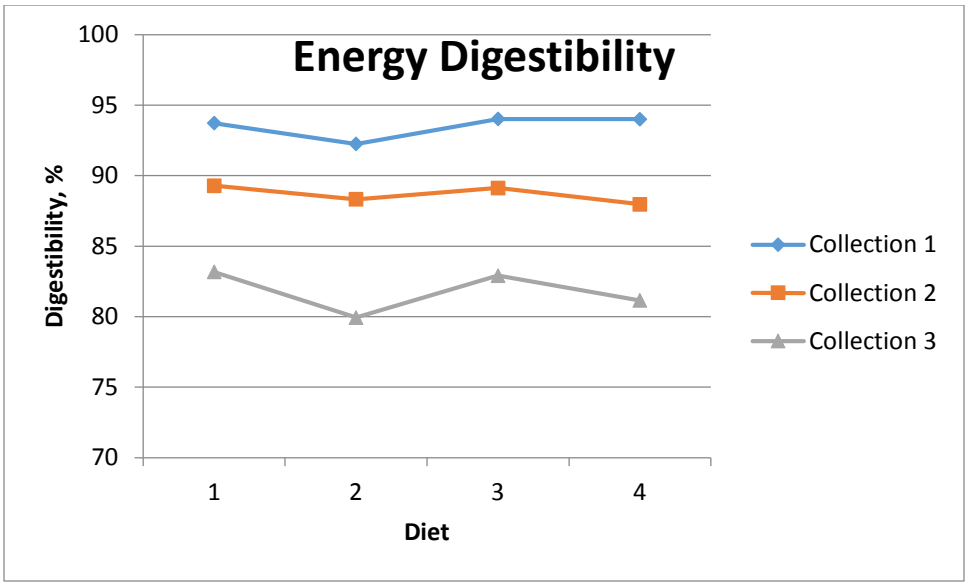


Figure 4.2 Effect of Naturally-Contaminated Corn on Energy Digestibility. Each mean represents 4 pens/treatment with 2 pigs/pen. The experimental period for collection 1 and 2 was 7 days, collection 3 was 5 days. Diets were complex nursery diets as follows: Diet 1 was a conventional corn-soybean meal, positive control (PC) diet; Diet 2 contained naturally-contaminated corn (NC diet); Diet 3 was a PC diet with sodium bentonite clay; Diet 4 was a NC diet with sodium bentonite clay. No diet effects ($P > 0.10$) or collection x treatment effects ($P=0.93$) were observed; collection effects were observed ($P<0.0001$).

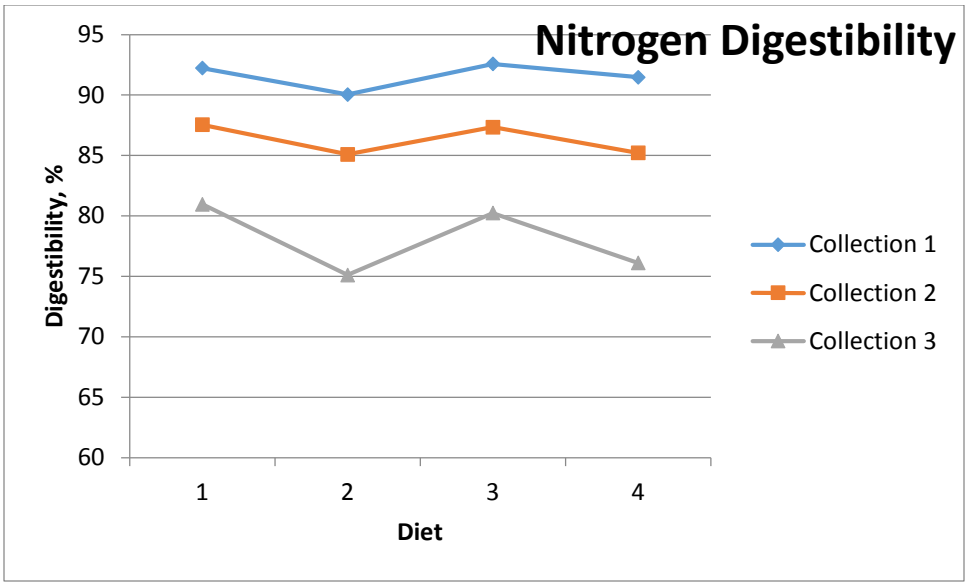


Figure 4.3 Effect of Naturally-Contaminated Corn on Nitrogen Digestibility. Each mean represents 4 pens/treatment with 2 pigs/pen. The experimental period for collection 1 and 2 was 7 days, collection 3 was 5 days. Diets were complex nursery diets as follows: Diet 1 was a conventional corn-soybean meal, positive control (PC) diet; Diet 2 contained naturally-contaminated corn (NC diet); Diet 3 was a PC diet with sodium bentonite clay; Diet 4 was a NC diet with sodium bentonite clay. Corn quality effects ($P < 0.05$) and collection ($P < 0.0001$) were observed; no collection x treatment effect ($P=0.86$).

Passage rate was affected by corn quality ($P=0.01$) with the contaminated diets seeing a longer period required for passage. Feed intake was affected by corn quality ($P<0.05$) with intake being increased in the diets not containing contaminated corn. Apparent dry matter digestibility was affected by corn quality as well ($P=0.02$) with digestibility increasing in the non-contaminated diets.

Energy digestibility, energy retention, digestible energy, and metabolizable energy ($P > 0.05$) were not affected by diet.

Nitrogen intake was affected by corn quality content ($P < 0.05$) with non-contaminated corn having a higher nitrogen intake. Nitrogen excretion was not affected

by corn quality, clay, or an interaction between the two. Nitrogen absorption and retention was affected by corn quality ($P<0.0001$) with more absorption and retention in the non-contaminated diets. Nitrogen digestibility was affected by corn quality ($P=0.01$). Nitrogen retention as a percent of intake was affected by the corn quality ($P <0.05$) as was retention as a percent of absorbed ($P=0.01$).

4.4. Discussion

Reduced feed intake due to contaminated feed was observed in this study which is similar to Harvey et al (1989), Escobar (2012), as well as Schell et al (1993) and Lindemann et al (1997). Unlike Edds (1979) and Escobar (2012), reduced growth performance due to corn quality was not noticed. Growth performance was similar to studies by Schell et al (1993) and Lindemann et al (1997) which showed that when clay was added to aflatoxin contaminated diets for weaning pigs, pigs were able to exhibit increased growth. With regards to preference, it was noticed that the pigs would root through the contaminated feed frequently as if searching for non-contaminated feed which is similar to Lindemann et al (1997). Rooting through the contaminated feed may have affected the recorded feed intake values as they would routinely push it out of the feeders. Significant feed rejection, vomiting and bloody feces were not noticed while feeding a combination of aflatoxin and FB_1 in comparison to diets that contain DON as well as aflatoxins or fumonisins (Harvey et al, 1989; Mirocha et al, 1978).

4.5. Conclusions

4.5.1. Experiment 1

This experiment showed that weaning pigs can and do show a preference to corn and soybean meal diets that are not contaminated by mycotoxins. When given the choice between two mycotoxin contaminated diets, the pigs showed a preference for the diet that contained added starch instead of added clay. It is unclear why the diet containing clay might be rejected but could be due to altered taste characteristics. There is also some chance that pigs wasted feed that they found unpalatable and thus the numbers are not entirely accurate. This experiment shows that even when fed a binding agent (clay), diets containing 0.03 ppm of Aflatoxins and 29.20 ppm Fumonisin B_1were undesirable. The findings in this research were very similar to a similar preference trial done by Escobar (2012).

4.5.2. Experiment 2

In this digestibility experiment, naturally-contaminated corn in diets with about 0.03 ppm of Aflatoxins (critical level in growing pigs is <0.1 ppm) and 29 ppm Fumonsin B_1(critical level in growing pigs is <10ppm), led to decreased feed intakes. Decreased feed intakes did not lead to the expected decreased growth performance parameters found in Escobar (2012) such as final weight and average daily gain, even though final weights were significantly different, the weights of pigs on good corn with starch and pigs on bad corn with clay were similar, as were the remaining two dietary treatments. Energy parameters including apparent energy digestibility, energy retention and metabolizable energies were not affected by dietary treatment although digestible energy was affected

and was lowest in the contaminated corn with clay diet. The most significantly affected digestibility parameters were that of nitrogen intake, absorption, retention, apparent nitrogen digestibility, as well as nitrogen retention as a percent of intake and nitrogen retention as percent of absorbed. The parameters were all higher in the non-contaminated corn diets than the contaminated diets, with the contaminated corn with clay diet being lowest of all. Ultimately, the diets with corn that were not contaminated with aflatoxins and fumonisins were more readily digestible than the diets with naturally-contaminated corn. The inconsistency among mycotoxin assays may have a significant effect on the resulting responses to feed exhibited by pigs.

Unfortunately mycotoxin research can be very inconsistent due to the large variability that occurs in feed contamination, feed sample collection, feed sample assays (see Table 4.2), and the ability of the pigs to overcome the contamination in the feed. As evidenced by the research above, as the pigs aged they were better able to compensate for the quality of the feed and achieve a similar growth and ADG as the pigs on the non-contaminated diets. Further research investigating the ability of pigs to overcome the combination of mycotoxins (especially aflatoxin and fumonisins) could help to serve the pig production system.

APPENDICES

Appendix 1. Effect of Supplementation of Enzymatically Hydrolyzed Yeast (EHY) on Digestibility in Finishing Pigs in Collection Period 1

		Treatment means[1]					Treatment P-values		
Response		1	2	3	4	PSEM	Lysine	EHY	Interaction
Performance									
	Initial Weight, kg	44.15	44.60	44.75	45.21	1.07	0.59	0.69	1.00
	Final Weight, kg	46.42	48.53	46.87	47.93	1.44	0.96	0.31	0.73
	ADG, kg/d	0.45	0.79	0.42	0.54	0.18	0.47	0.25	0.57
DM									
	Passage Rate (hours)	36.08	36.00	38.50	35.25	5.55	0.89	0.77	0.79
	Intake, kg/d	1.05	1.07	1.06	1.05	0.02	0.95	0.62	0.38
	Digestibility, %	90.83	89.27	90.15	89.88	0.60	0.95	0.18	0.32
Energy									
	Apparent digestibility, %	90.74	89.41	90.01	89.96	0.63	0.89	0.32	0.35
	Retention, % of intake	88.20	87.02	87.87	87.81	0.69	0.75	0.40	0.44
	Retention, % of absorbed	97.21	97.32	97.62	97.61	0.22	0.16	0.81	0.79
	Digestible Energy (kcal/kg of intake)	3566.13	3514.14	3525.63	3523.70	24.76	0.56	0.32	0.35
	Metabolizable Energy (kcal/kg of intake)	3466.62	3420.06	3441.71	3439.70	26.98	0.93	0.40	0.44
Nitrogen									
	Intake, g/d	31.18	32.00	29.76	29.54	0.53	0.01	0.59	0.36
	Excreted in urine, g/d	11.19	12.93	10.51	12.40	1.75	0.74	0.34	0.97
	Excreted in feces, g/d	3.40	4.02	3.39	3.31	0.23	0.18	0.29	0.19
	Total excreted, g/d	14.59	16.94	13.90	15.71	1.62	0.57	0.25	0.87
	Absorption, g/d	27.78	27.98	26.37	26.22	0.52	0.02	0.96	0.75
	Retention, g/d	16.59	15.06	15.86	13.83	1.59	0.56	0.31	0.88
	Digestibility, %	89.14	87.46	88.56	88.86	0.77	0.61	0.41	0.25
	Retention, % of intake	53.26	47.23	53.94	47.04	4.89	0.96	0.23	0.93
	Retention, % of absorbed	59.78	54.22	60.97	52.86	5.89	0.99	0.29	0.84

[1] Values represent 12 pigs per treatment, with Diet 1 was a conventional corn-soybean meal, positive control (PC) diet meeting the SID lysine need (NRC, 1998) with no EHY; Diet 2 was a PC diet with 0.2% EHY; Diet 3 was a negative control diet (NC) with a 9% reduction in SID lysine with no EHY; Diet 4 was an NC diet with 0.2% EHY.

[2] PSEM-Pooled Standard Error of the Mean

[3] Collection Period was 7 days.

Appendix 2. Effect of Supplementation of Enzymatically Hydrolyzed Yeast (EHY) on Digestibility in Finishing Pigs in Collection Period 2

		Treatment means[1]					Treatment P-values		
Response		1	2	3	4	PSEM	Lysine	EHY	Interaction
Performance									
	Initial Weight, kg	55.19	57.00	57.30	57.76	1.21	0.28	0.39	0.59
	Final Weight, kg	64.11	64.56	65.47	64.11	1.36	0.75	0.75	0.53
	ADG, kg/d	1.78	1.51	1.63	1.27	0.23	0.43	0.22	0.85
DM									
	Passage Rate (hours)	46.83	38.33	48.00	49.67	3.71	0.14	0.39	0.22
	Intake, kg/d	1.35	1.36	1.37	1.39	0.03	0.38	0.74	0.84
	Digestibility, %	89.13	89.01	89.39	89.99	0.67	0.39	0.73	0.61
Energy									
	Apparent digestibility, %	88.87	88.79	89.09	89.91	0.61	0.31	0.57	0.49
	Retention, % of intake	87.08	86.79	87.32	88.06	0.55	0.22	0.70	0.38
	Retention, % of absorbed	97.99	97.75	98.01	97.94	0.10	0.33	0.18	0.43
	Digestible Energy (kcal/kg of intake)	3496.37	3493.13	3490.81	3523.04	24.03	0.63	0.57	0.49
	Metabolizable Energy (kcal/kg of intake)	3425.96	3414.43	3421.20	3450.46	21.75	0.50	0.70	0.38
Nitrogen									
	Intake, g/d	41.31	41.46	37.49	37.88	0.75	0.00	0.73	0.88
	Excreted in urine, g/d	9.52	12.24	9.67	11.13	0.75	0.55	0.03	0.44
	Excreted in feces, g/d	4.84	4.72	4.50	4.35	0.33	0.32	0.69	0.96
	Total excreted, g/d	14.36	16.96	14.17	15.48	0.85	0.37	0.06	0.48
	Absorption, g/d	36.48	36.75	33.00	33.53	0.58	0.00	0.52	0.83
	Retention, g/d	26.95	24.51	23.32	22.40	1.08	0.04	0.17	0.51
	Digestibility, %	88.30	88.63	88.02	88.51	0.72	0.78	0.58	0.91
	Retention, % of intake	65.13	59.10	62.01	59.09	2.26	0.51	0.10	0.52
	Retention, % of absorbed	73.71	66.66	70.49	66.76	2.41	0.54	0.07	0.52

[1] Values represent 12 pigs per treatment, with Diet 1 was a conventional corn-soybean meal, positive control (PC) diet meeting the SID lysine need (NRC, 1998) with no EHY; Diet 2 was a PC diet with 0.2% EHY; Diet 3 was a negative control diet (NC) with a 9% reduction in SID lysine with no EHY; Diet 4 was an NC diet with 0.2% EHY.

[2] PSEM-Pooled Standard Error of the Mean

[3] Collection Period was 7 days.

Appendix 3. Effect of Supplementation of Enzymatically Hydrolyzed Yeast (EHY) on Digestibility in Finishing Pigs in Collection Period 3

		Treatment means[1]					Treatment P-values		
Response		1	2	3	4	PSEM	Lysine	EHY	Interaction
Performance									
	Initial Weight, kg	68.64	71.06	72.73	71.37	1.81	0.11	0.67	0.16
	Final Weight, kg	75.90	76.81	77.72	76.51	1.40	0.61	0.92	0.48
	ADG, kg/d	1.45	1.15	1.00	1.03	0.13	0.08	0.35	0.26
DM									
	Passage Rate (hours)	47.00	40.67	45.83	46.00	6.64	0.76	0.66	0.64
	Intake, kg/d	1.63	1.67	1.71	1.64	0.03	0.37	0.72	0.12
	Digestibility, %	91.57	89.92	90.55	90.50	0.81	0.80	0.33	0.36
Energy									
	Apparent digestibility, %	91.16	89.78	90.19	90.38	0.79	0.82	0.48	0.36
	Retention, % of intake	88.81	87.50	87.94	88.11	0.77	0.87	0.48	0.37
	Retention, % of absorbed	97.42	97.45	97.51	97.49	0.09	0.53	0.93	0.82
	Digestible Energy (kcal/kg of intake)	3597.14	3542.73	3507.04	3514.15	30.85	0.10	0.47	0.36
	Metabolizable Energy (kcal/kg of intake)	3504.36	3452.52	3419.63	3426.04	30.02	0.11	0.48	0.37
Nitrogen									
	Intake, g/d	48.66	49.98	49.17	47.23	0.91	0.27	0.74	0.12
	Excreted in urine, g/d	17.26	21.77	20.35	17.63	1.31	0.70	0.52	0.03
	Excreted in feces, g/d	5.00	5.95	5.61	5.32	0.48	0.98	0.52	0.24
	Total excreted, g/d	22.26	27.72	25.96	22.95	1.55	0.74	0.46	0.03
	Absorption, g/d	43.66	44.03	43.56	41.91	0.87	0.25	0.49	0.29
	Retention, g/d	26.40	22.26	23.21	24.28	1.43	0.70	0.32	0.12
	Digestibility, %	89.77	88.10	88.59	88.84	0.98	0.83	0.49	0.36
	Retention, % of intake	54.49	44.68	47.10	51.84	2.99	0.97	0.43	0.05
	Retention, % of absorbed	60.65	50.73	53.18	58.21	3.04	1.00	0.45	0.05

[1] Values represent 12 pigs per treatment, with Diet 1 was a conventional corn-soybean meal, positive control (PC) diet meeting the SID lysine need (NRC, 1998) with no EHY; Diet 2 was a PC diet with 0.2% EHY; Diet 3 was a negative control diet (NC) with a 9% reduction in SID lysine with no EHY; Diet 4 was an NC diet with 0.2% EHY.

[2] PSEM-Pooled Standard Error of the Mean

[3] Collection Period was 7 days.

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