

THESIS

THE EFFECTS OF HEMPSEED CAKE ON SWINE BEHAVIOR

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ABSTRACT

THE EFFECTS OF HEMPSEED CAKE ON SWINE BEHAVIOR

In recent years, research using hemp has become more frequent due to the increasing number of states opting to legalize the production of this agricultural product. With increased production, comes an increase in the amount of *Cannabis sativa L.* byproduct generated. This leaves the industry looking for a sustainable solution to this novel feedstuff and outlet to distribute this material. Previous research efforts have evaluated the impact of this additive on the quality of the animal byproducts in the form of hempseed cake, however, a dearth of knowledge exists regarding how this additive impacts behavior and subsequent welfare. This study compared an experimental group of pigs fed a hemp diet to a control group fed a soybean diet. The behavior and signs of stress were recorded and compared between the two groups and used to determine if the experimental feed altered the behavior of animals that were reared in the same conditions as the control group. The goal of this study was to investigate how the inclusion of hempseed byproducts into the diet of finishing pigs impacted behavior and their responsiveness to novelty. Overall, there were limited significant findings to determine that the inclusion of hempseed cake as a feed additive altered the pigs' behavior. One notable finding was that during the human approach test the treatment group had reduced overall activity, which aligns with reported side effects of consuming hemp in various forms in other species. For the scale test, there was an observable trend of an increase in lower scores with each test performed, except for day 60 which deviated from this trend.

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PREFACE

Pork accounts for 36% of the meat consumed in the world, making it the number one consumed meat product worldwide (Agriculture and Consumer Protection Department: Animal Production and Health, 2014). For the pork industry to meet consumer demands, pork production must be as efficient as possible. With a product already in high demand, combined with the projected 9.8 billion world population by 2050 estimation, the pork industry is under pressure to increase output, while using fewer resources (United Nations, 2017). This phenomenon is known as the “Food Crisis of 2050” and refers to the fact that food production by 2050 must be twice as productive as it is today (United Nations, 2009). As such, researchers are highly dedicated to finding innovative ways to approach this issue.

Feed costs account for over two-thirds of the total cost of running a swine farm. These costs stem from the cost of the feed itself, as well as the rate of conversion (Lammers et al., 2007). Producers are continually searching for ways to utilize resources efficiently and evaluate the feasibility of new feedstuffs. With the increased availability of hempseed byproduct in recent years, it raises the question of if it could serve as a protein source for livestock. Hempseed may have the potential to replace more costly protein sources, such as soybean, by means of yielding a product with comparable, or superior, meat quality compared to the traditional soybean feed diet (Fike, 2016; Parr et., 2020). By being able to produce a more cost-efficient product, producers could have the ability to become more competitive in the market. Current published research in this area has been solely focused on the effect of the diet on the byproducts of the animal. This is important because it gives producers an idea of how it will affect their output, as well as the safety implications of utilizing it. The effect on output is important information for

producers to have because it affects how much product they can sell, and therefore their bottom line. However, safety is always a number one concern because if a product is not safe, it can have disastrous effects on animal welfare, consumer health, profits, and even result in legal consequences. While this is an important area of interest, for a new feedstuff to be socially sustainable, there is a need to evaluate the impact of diet on the welfare and behavior of the animal that is consuming this new product. This literature review will investigate how the effects of using hempseed cake as a protein source in swine feed impacts swine behavior and productivity.

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CHAPTER 1- LITERATURE REVIEW

HEMP

Cannabis sativa L., commonly known as “hemp” is a versatile plant that is believed to have come from Eurasia to North America in the year 1606 by British settlers in Jamestown. While some may associate this plant as being the same thing as marijuana, there is a distinction between the two. They both derive from the *Cannabis sativa* plant yet differ in the amount of cannabidiolic acid (CBDA) and Tetrahydrocannabinol (THC) they contain. THC is the psychoactive component in marijuana that produces the “high” feeling that is typically associated with cannabinoid use (Gaoni & Mechoulam, 1964; Fernandez & Abdi, 2009). However, hemp contains far less THC than marijuana and therefore would not produce the same side effects.

Traditionally, hemp has been used in home remedy medications, food, feed additives, skin moisturizers, wood preservatives, soap, ink, and detergents (West, 1998; Oomah et al., 2001). As technology became more advanced, so did the utilization of hemp. Today, there are more than 25,000 products made from hemp worldwide. They are categorized into food and beverages, textiles, furniture, paper, personal care, construction materials, automotive, recycling, and agriculture. In agriculture, it can be used as a feed additive, rotational crop, or cover crop (Johnson, 2018). Primarily used as a fiber source, the hemp industry was brought to a halt in the United States in 1970 by the Comprehensive Drug Abuse Prevention and Control Act. This Act is comprised of Title II and Title III and is enforced by The Drug Enforcement Agency. Title II was also known as the Controlled Substances Act and started what the government called the

“war on drugs.” This Act addressed the distribution and manufacturing of narcotics, steroids, hallucinogens, and main ingredients of controlled substances. The Controlled Substances Act also created a classification system to categorize the based-on use or risk for potential abuse. Schedule I drugs are drugs that have the highest risk for dependency or abuse. They include heroin, peyote, LSD, ecstasy, and marijuana. This Act also labeled hemp as a Schedule I controlled substance and therefore affected its ability to be utilized (Cherney & Small, 2016). Schedule II drugs are considered less dangerous than Schedule I and are comprised of Ritalin, cocaine, Adderall, meth, OxyContin, methadone, and Demerol. Schedule III drugs are less addicting than Schedule II and is made of testosterone, Vicodin, anabolic steroids, Tylenol with codeine, and ketamine. Schedule IV drugs have a small chance of developing dependency and are Ambien, Xanax, Ativan, Soma, Valium, Darvon, and Darvocet. Schedule V drugs have the least potential for dependency and include medicines such as Parepectolin, Lomotil, Lyrica, Motofen, and cough syrup with codeine. Title III of the Comprehensive Drug Abuse Prevention and Control Act put laws in place to control how drugs are imported, exported, and drug confiscation (FindLaw, 2019). However, in 2014 a Farm Bill was introduced which allowed for specific entities to cultivate hemp for research (Johnson, 2021). The use of hemp then became more frequent due to the 2018 Farm Bill which removed hemp from the Controlled Substance Act, as well as classifying it as an agricultural product. The 2018 Farm Bill also formally declared it legal, under certain restrictions, including being subject to state law, as well as further defining it as a product separate from marijuana.

Currently, The USDA is responsible for regulating hemp production. While certain states may not have legalized hemp, this bill legalizes the transport of it through all states, regardless of the specific state’s laws (DuBois & Bridges, 2020). While the 2018 Farm Bill has led to many

new discoveries regarding hemp, their implementation into the marketplaces has been slow and challenging due to the lack of evidence-based research, lack of regulation, and improper labeling (Vandrey et al., 2015). As of 2021, studies have been conducted by several countries to establish a safety threshold for the daily recommended amounts of hemp, however, no such studies have definitively established these recommendations in livestock or pets (Cital et al., 2021).

The two main byproducts from hempseeds are the oil that is pressed from seeds and the hempseed hull. The hempseed, or grain, is high in nutrients and low in cannabinoid content and contains omega-3 and omega 6 fatty acids, Gamma-linolenic acid, and protein. These seeds can be used as a whole, or the hulls can be taken apart and used for their fiber content, protein, or oil (Cital et al., 2021). Hempseed cake, the product that is used as a feed additive, is a product that results from using the remaining fiber, proteins, and biomass from the cold pressing procedure that extracts the oil from the seed (Callaway, 2004).

The protein in hemp is nutritionally comparable to that of other nut proteins, such as soybean and egg white, therefore contributing towards the compelling argument to use hemp byproduct as a replacement for soybean feed. As a feed additive, hempseed cake meets the protein needs of livestock such as poultry, swine, cows, and fish. European standards recommend a protein inclusion rate of 3% for broilers, up to 7% in laying hens, and up to 5% for pigs, fish, and cows. (EFSA, 2011). According to the USDA Nutrient Database list, the seeds from hemp are rich in vitamins E, C, A, thiamine, riboflavin, niacin, folate, and riboflavin (Galasso et al., 2016). Hempseeds are also rich in several minerals such as calcium, manganese, sodium, zinc, phosphorus, as well as iron, and does exceptionally well in absorbing minerals found in the soil surrounding where it grows (Mihoc et al., 2012). Compared to the hemp plant's fat and protein content, it has a relatively small amount of carbohydrates, which are mostly

comprised of fiber that are found in the hull. The ratio of water soluble and water insoluble fiber is 20:80 (Leonard et al., 2019).

The hemp plant does contain several anti-nutritional factors, such as phytate and trypsin, that can impede the digestibility of the proteins for nonruminant animals (Galasso et al., 2016). As of 2022, 37 states, the District of Columbia, and four territories have legalized medical marijuana, and 19 states, the district of Columbia, and two territories have legalized recreational marijuana (Hanson, 2022). The market for cannabis products realized a 74% market growth in 2014, making it a \$1.5 billion industry in 2013. It is one of the greatest expanding markets existing in the U.S.A (Skodzinski, 2015). Over 23 U.S. states, as well as Washington D.C., have introduced laws that allow for the medicinal use of marijuana and hemp (PDQ, 2022). Other states, such as Colorado, Washington, Alaska, and Oregon have legalized the recreational use of cannabis products (Ausick, 2015). However, due to hemp being formerly regarded as a controlled substance and classified as a Schedule I agent, research involving it has been restricted in past years due to the legal nature of its use in certain states. Therefore, its effects on animals and humans have yet to be fully understood by way of clinical trials (Nolan, 2013; Robinson, 2014). Due to the lack of published research on how hempseed cake affects the behavior of swine, researchers must speculate on how behavior will be affected by it. However, due to the biological similarities shared by humans and swine, researchers can hypothesize what behavior changes may occur by studying the effects in humans.

Hemp Use in Humans

Humans consume hemp in many forms and while published research on the behavioral effects of hemp is limited, there are several health conditions that are believed to be alleviated by using it. These conditions include eczema, psoriasis, arthritis, pain management, weight loss,

digestive issues, hair loss, dandruff, itchy scalp, post menstrual symptoms, fetal development, inflammation, cancer, high cholesterol, memory loss, anxiety, depression, fatty acid deficiency, and compromised immunity (Hall, 2019). Hemp consumption was observed to alleviate symptoms of muscle tension, pain, vomiting from chemotherapy, weight gain tendency in HIV patients, Tourette's, and sleep disturbance. However, feeling disoriented, muscle weakness, trouble keeping balance, diarrhea, sleepiness, dry mouth, and hallucinations were all reported side effects (Whiting, 2015; PDQ, 2022). Now that the effects of hemp are somewhat understood in humans, it is important to justify why these observations are important when evaluating the effects of hempseed cake in swine.

BIOLOGICAL SIMILARITIES BETWEEN SWINE AND HUMANS

Humans and swine are morphologically similar such that they are commonly used as models for medical research and are similar to humans regarding their heart function (Swindle & Smith, 1998; Swindle, 2007).

The swine integument system is also biologically similar to humans and is commonly used in medical research as a model for human skin. A pig's skin is comparable in thickness, as well as structure and has a similar hair distribution and hair follicle structure. Pigs are also used in wound healing experiments due to their similarity with humans in blood flow and reepithelialization (Hartwell, 1955; Swindle & Smith, 1998). The dimensions and genetic makeup of the swine brain have led to increased interest in using swine as a model for the central nervous system humans (Lind et al., 2007). And finally, swine have seven extraocular muscles with a retina, pupil, a comparable vitreous humor, and open field vision, which are also similar humans (Swindle, 2007; Swindle et al., 2008).

HEMP USE IN PETS

Not only has hemp penetrated the consumer market, but its use has also gained interest in the pet market as well. It is sold in the form of treats, oil, and capsules. Pet owners claim that they have noticed improvement in their animals when given to treat seizures, pain, arthritis, mood stabilization, and appetite enhancement. One study by the AVMA found that pet owners are also distributing these products to improve symptoms of separation anxiety, IBS, nausea, and noise phobia (Noland, 2013). However, research in this field is limited and veterinarians, as well as pet owners, have little scientific evidence to base treatment plans on (Robinson, 2014). One 2016 study from Colorado State University conducted a survey for pet owners who distributed hemp products to their animals and recorded their findings. They discovered that out of the dog owners who participated in the study, 64.3% indicated moderate improvement in symptoms of pain, 50.5% said it improved their dogs sleeping habits, and 49.3% reported a moderate improvement in anxiety symptoms. The most common side effects of the drug included an increased calming effect (22%), and increased appetite (15.9%).

The responses from cat owners indicated that 66% felt there was moderate improvement in pain, 56.3% indicated a moderate improvement in inflammation, and 44% indicated improved sleep quality. Increased calmness (19.2%) and increased appetite (16.2%) were also the two most reported side effects. When respondents were asked how they felt about the effectiveness of the product, 62.48% responded that they felt hemp worked better than other treatments (Kogan et al., 2016). Now that some data has been collected regarding how hemp products affected pets, it is important to use this information, combined with the existing information on the effect it has on

livestock, when speculating the effects it may have on the wellbeing of various species that were exposed to hemp byproduct.

HEMP RESEARCH IN NON-PORCINE LIVESTOCK

Poultry

Omega 3 and omega 6 play an important role in immunity, growth, metabolism, performance, reproductive, and product quality regarding their effect on poultry. Due to hemp's nutritional content in this area, the poultry industry has taken an interest in further investigating its effects (Alagawany et al., 2019). Not only are producers interested in the omega 3 and omega 6 contents in hempseed cake, but an added benefit of utilizing it in poultry feed is that it also contains glycerides of lauric acid (GLA) and alpha-lipoic acid (ALA), which can be converted to docosahexaenoic acid (DHA). DHA has shown to promote growth, increase fertility, improve immunity, and increase bone strength in poultry and swine. This could result in a hardier, healthier animal, that could lead to improvements in welfare due to a decrease in illness (Lee et al., 2018).

Studies show that obtaining the perfect hempseed ratio in feed is very important. For example, one study fed laying hens a 30% dehulled hempseed diet and experienced a 30% decrease in egg weight that was thought to be caused by the neutral detergent fiber content of the feed. When fed 4.5% or 9% hempseed oil, or up to 20% hempseed in the diet, egg production, feed intake, and carcass weight was not impacted. However, laying hens consuming less than 4% hemp oil did experience decreased feed intake, but this did not translate into decreased laying performance (Goldberg et al., 2012; Neijat et al., 2014).

In addition to ratio, it appears that delivery also plays a part in the effect of hempseed cake. Another study showed that hens fed 15% raw dehulled heated hempseed compared to a comparable amount of hempseed that was heated for one hour at 338 °F, yielded increased egg weights as well as increased linoleic acid (LA), ALA, and DHA amounts found in the eggs and did not appear to affect egg quality (Konca et al., 2019). Pending the results of current research, it appears that 20% hempseed or 9% hempseed oil appears to be the optimal hemp ratio in poultry feed to optimize the nutritional value of omega 3 found in the eggs of hens fed this diet. Furthermore, several studies also found evidence to support these numbers. When ratios of hemp byproduct were increased in the feed, they discovered that although omega 3 levels were increased in the eggs, DHA, eicosapentaenoic acid (EPA), and ALA levels all fell below recommended levels (Silversides & Lefrancois, 2005; Goldberg et al., 2012; Neijat et al., 2014; Park et al., 2014; Konca et al., 2019).

Regarding broiler chickens, current research suggests the feed conversion rate to be 2.5-20% in traditional feed rations, and that utilizing whole seeds vs. dehulled seeds has an impact on feed conversion rates (Št'astník et al., 2016; Jing et al., 2017). Utilizing hemp byproduct as a feed additive not only increases omega 3 levels in the eggs of laying hens, but it also increases the omega 3 levels in the meat when fed to broiler chickens, as well as ducks, quail, and pheasants (Yalcin et al., 2018).

Ruminants

Due to the differences between a monogastric digestive system and a ruminant digestive system, the benefits of feeding a hempseed cake ration to a ruminant are much more complicated to ascertain. Due to the transformation of proteins and fatty acids in the rumen digestive system, the benefit of having the increased omega 3 content in the hempseed cake feed ration is not as directly transferred to the products of a ruminant as it is with a nonruminant. In addition to the questionable nutrient content of the byproducts, ruminant health also comes into question when feeding hemp in certain forms. For example, feeding hemp as a whole product to a ruminant would result in an exceptionally high NDF rate, which would translate into adverse effects on the ruminant digestive system. However, these effects are minimized when fed as in a dehulled form or as a hempseed cake.

Very little research has been conducted to discover the impact of a hemp diet on milk production and quality. The Cannabis Therapy in Veterinary Medicine guide states that the low inclusion rate of hemp makes corn silage or haylage a far superior feed than hemp when it comes to milk production (Cital et al., 2021). However, one study involving 40 Red Swedish cows concluded that feeding 143 g/kg of hemp in the feed resulted in not only better yields, but fat, lactose, and protein content of the milk as well (Karlsson et al., 2010).

Equine

Administering cannabis products to horses is a practice that dates back to the Ancient Greeks (Butrica, 2008). The American Veterinary Medical Association (AVMA) even reported in 1913 recommendations for *Cannabis indica* use in horses (Lyman, 1913). It is difficult to trace the use of hemp during this time because *Cannabis sativa*, the plant that hemp derives

from, was referred to as *Cannabis indica*. Therefore, there would be no way to distinguish a history of hemp, CBD, or marijuana use in the equine industry via AVMA guidelines. The authors of The Cannabis Therapy in Veterinary Medicine handbook claim to have administered up to 3000 mg/day of hemp to patients and observed no ill effects. While cannabis administration to equines is not a new concept, it is not as strictly regulated in horses due to the fact that horses are largely a companion animal. Therefore, cannabis use is not restricted to solely hemp. Consumers are utilizing CBD as well as THC products as a means of treatment for their animals.

HEMP RESEARCH IN SWINE

Several studies have been conducted to better understand the impact of feeding hempseed cake to pigs. However, they are all strictly focused on how the diet affects pork products rather than anything behaviorally or welfare related. With the current state of research, it can only be speculated what side effects the pigs will experience based on the side effects that have been reported in humans and pets. For example, hemp is often used as a pain reliever and as an anti-inflammatory. This could possibly mean that the feed additive could improve lameness, which can take pigs out of production for costly treatment. Animals that cannot walk, cannot go to slaughter, and therefore must be humanely euthanized at the cost of the producer. Previous research also indicates that hempseed cake can strengthen the skeletal system of poultry and swine, therefore adding value to this hypothesis (Lee et al., 2018). If the side effects of pain management are as effective in pigs as they are in humans, then one could also speculate that it could relieve arthritis in older animals such as breeding sows and teaser boars.

Chronic pain of any kind is considered a welfare issue. Therefore, any novel methods to prevent or manage this symptom should be explored, especially if it can be done effectively and not disturb farm operations. In addition to pain management, hemp has also been used to reduce symptoms of anxiety and depression in humans. Pigs are very intelligent and inquisitive animals who crave stimulation and companionship and can often develop stereotypical behaviors due to lack of stimulation. Examples of these can be rooting, vacuum chewing, belly nosing, or tail biting, and can be a sign of mental distress. If hemp intake in humans has shown an anti-anxiety

effect, one might speculate that it could also have a calming effect on the mental state of the pigs and therefore reduce stereotypical behaviors.

Hemp has also shown to boost immunity and prevent illness (Hall, 2019). While pigs are vaccinated for various illnesses, colds, flu, porcine epidemic diarrhea virus (PED), and porcine reproductive and respiratory syndrome virus (PRRS) can still occur and cause physical distress to the animal, as well as financial stress to the producer. Due to the devastating effects these illnesses can have on production and animal welfare, any means of boosting immunity would be welcomed by the industry. However, several negative side effects of hemp use have been reported as well. These include dry mouth, diarrhea, vomiting, confusion, difficulty keeping balance, and hallucinations (Whiting, 2015; PDQ, 2022). Dry mouth, vomiting, and diarrhea can all be signs of dehydration and a failure to thrive. In an experiment, these symptoms must be monitored to ensure animal health. Changes in water intake could signal to the producer that their animals might be drinking more or less than normal and utility expenses may fluctuate. If the animals did experience hallucinations and had trouble keeping balance, that would also be considered a welfare issue.

As of now, none of the symptoms experienced by humans have been documented to also exist in swine due to lack of research in this field. However, it is important to use all available information when trying to assess the acceptability of any new feed additive. As far as established research goes, studies suggest a 28% hempseed cake feed ration is the maximum allowable ration that will not impede digestion of fat, protein, and other feed content (Presto et al., 2011). One study that focused on the effects of feeding hempseed cake to farrowing sows found that the nutritional makeup of their colostrum and milk was enhanced compared to the control group. This led to enhanced piglet thriftiness, weight, and overall health (Hăbeanu et al.,

2016). Another study by Aarhus University also corroborated these results in a similar study. They found that hempseed cake inclusion in a sow diet enhanced milk in a way that was beneficial for piglet health, as well as weight gain (Vodolazska & Lauridsen, 2020). Additionally, the National Research Development Institute for Animal Biology and Nutrition found evidence that feeding hempseed cake to sows during late gestation and lactation, as well as in creep feed for piglets, resulted in not only improved oxidative status for both sows and piglets, but enhanced antioxidative status markers. As a result of these two findings, the study concluded that the inclusion of hempseed cake allowed for increased health of the sow and offspring, as well as a less stressful transition during weaning (Palade et al., 2019).

Although hemp has shown promising results in various studies across livestock species, as well as in self-reported use in humans and pets, the swine industry is in an optimal position to potentially maximize from the benefits that it could have to offer based on the reported outcomes of its use. For example, because feed expenditures account for over 2/3s of production cost, they are the number one expense on a swine farm (Lammers et al., 2007). Due to the legalization of hemp and increased interest in the cannabis market, production rates are increasing, as well as the amount of by product produced. Currently, there is no market for the byproduct, so the industry is looking for markets to sell to. Because of this, no exact price point has been established, however, the swine industry is always looking for ways to decrease production costs. Previous research has shown promising results in using this byproduct as a feed additive. Therefore, if hempseed cake proves to be an affordable protein source, then it could very well serve as a means to manage production costs, without comprising the product. However, further research on the welfare effect of this additive, as well as the safety ramifications of it should be explored prior to implementing its use.

Due to the heightened interest in the cannabis field, as well as the legalization of hemp, combined with the needs of the pork industry, the need for evidence-based research to find out exactly how hempseed cake affects the wellbeing and behavior of swine is of the utmost importance. Continuing to investigate the effects of hempseed cake through research will ensure that all variables are known, so that the industry can move forward in an economically sustainable and welfare minded fashion.

Effects of Fiber in the Swine Diet

Hemp has a relatively high fiber content compared to the nutritional needs of swine. The seeds from the hemp plant contain 28% total dietary fiber and hemp cakes typically contain 42.6% dietary fiber (Callaway, 2004; Wang et al., 2013; Radočaj et al., 2014). In large scale farming productions, soybean and corn are the biggest components of the swine and poultry diet (Leeson, 2012). However, in a family farm setting, especially in countries such as Asia, more accessible high fiber feedstuffs such as brewers' grain, cassava leaves, water spinach, and rice bran may be used as feed. When swine are fed a high fiber diet, it can pose a challenge to producers to ensure they are feeding it in proper quantities due to the nature of the monogastric animals' ability to digest bulky feed and ferment it (Bach Knudsen et al., 2012). When fed in improper quantities, it can lead to issues absorbing nutrients, as well as decreased net energy values (Noblet & Le Goff, 2001). While the amount of fiber included in the swine diet should be fed in moderation, it does serve an important purpose in maintaining homeostasis, particularly with proper digestion (Wenk, 2001). It has been found that adding fiber to the diet of sows fed ad libitum in either pens or stalls, reduces stereotypical behaviors while also giving the animal an opportunity to express natural behaviors such as chewing and rooting (Whittaker et al., 1998; Meunier-Salaün et al., 2001; O'Connell, 2007). Reproductive performance is also positively

correlated with a high fiber diet and can result in increased litter size, more feeding bouts, and a decrease in stereotypical behaviors performed by pregnant sows (Wittman, 1986; Fahey et al., 2001; Meunier-Salaün et al., 2001; Robert et al., 2002)

Including fiber in the diet can stimulate the gut, increase gut fill, and improve animal welfare (Wenk, 2001; De Leeuw et al., 2008; De Lange et al., 2010; Bach Knudsen et al., 2012). Welfare is thought to be improved by including fiber in the diet because an increase in gut fill contributes to satiety and is shown to be related to a decrease in post feeding stereotypical behaviors that are often expressed in animals that are raised in environments with little to no enrichment (Terlouw et al., 1991; Brouns et al., 1994; Bergeron et al., 2000; Ramonet et al., 2000). Satiety has also been shown to be correlated to the amount of physical activity the animal engages in. Swine that are on restrictive feeding schedules have been shown to exhibit an increase in activity or rooting behaviors immediately following a meal, as well as before their next scheduled feeding time (Beattie & O'Connell, 2002). This is thought to be in preparation for their next meal and therefore an indicator of the level of fullness they experienced from the feed they ate in their morning feeding. Feeding frequency and time between engaging in bouts of feeding are also indicators of satiety (De Leeuw et al., 2008).

Due to the structure of fiber, it can cause the feed to increase in bulk and increase water holding capacity. This can lead to a decrease in motivation to feed because of the increase in chewing time, which stimulates mechanoreceptors in the gut, which then causes satiation. When this happens, the pig will stop feeding and reduce its feed intake (Paintal, 1954; Rolls & Rolls, 1997; Cummings & Overduin, 2007). The origin, composition, and type of fiber used can have varying effects on the individual ingesting it. For example, insoluble fiber can increase digestion time and the bulk of fecal matter. Soluble fiber can lead to an increase in density and moisture

content (Bach Knudsen, 2001). In addition to fiber type, the size of the particles in the feed can alter the impact of the feed as well. One study involving Yorkshire X Landrace crosses showed that reducing the particle size of the feed from three mm to one mm can lead to an increase in digestibility, as well as average daily gain in the post weaning stage of life. However, this effect did not carry into the grower phase (Ngoc et al., 2011). Age and breed of the pig can also impact the digestion of fiber as well. Studies show that older animals are more capable of digesting fiber due to an increased development of the gastrointestinal tract, decreased feed intake, and decreased gut motility (Shi & Noblet, 1993). Breeds such as the Mong Cai have been shown to have an advantage in digesting fiber due to the decreased digestion time which leads to an increased contact time between the digesting feed, enzymes that aid in digestion, and gut microbiota. This leads to increased absorption of nutrients and better digestion (Freire et al., 2000; Ngoc et al., 2013).

ANIMAL WELFARE

Animal welfare is a very important aspect of the farming industry and is enforced through various means. The basis for animal welfare in any animal related setting, stems from the Brambell Report, which was created in 1965, and was later morphed into the Five Freedoms by the Farm Welfare Council. The Five Freedoms are meant to provide a basic guideline for keeping animals in an environment that satisfies the minimum requirements for animal welfare. The Five Freedoms are: freedom from hunger and thirst, discomfort, pain/injury/disease, fear and distress, and freedom to express normal behavior (Farm Animal Welfare Counsel, 2009). When implementing any new farm practice, these are the parameters that researchers must compare their methods to when deciding if the new practice is acceptable or not. Any practice that does not abide by these Five Freedoms, compromises the animal's welfare and quality of life. Due to the lack of previous research regarding how hempseed cake affects the welfare and behavior of pigs, it is important that the Five Freedoms be referred to when reviewing research results.

Stress

When being faced with a stressor, an animal goes through three stages. They are recognition, biological defense, and the consequences of having faced the stressor (Moberg, 1985). The "consequences" stage is the most significant of the three stages because it determines how severely the stressor will impact the animal's wellbeing. The "recognition" stage of the stress response starts in the central nervous system when the perceived stressor is seen as a potential upset to homeostasis. No matter how dangerous the stressor actually is, the animal's perception of the stressor is key to how this stage begins. This is why seemingly trivial things,

like being separated from the herd temporarily, can have such an effect on certain species. No real danger is present, but the animal perceives there to be (McEwen & Stellar, 1993).

Once this process is initiated, a four-stage response is set forth by the central nervous system. These stages consist of the behavioral response, the autonomic nervous system response, the neuroendocrine response, and the immune response. The most critical stage is the behavioral response because it can help the animal avoid a potentially dangerous situation by simply removing itself from the presence of the stressor. This would be helpful in situations such as a predator, unfavorable weather conditions, or a cannibalistic herd mate. However, the animal does not always have the option to simply remove itself from the presence of the stressor, so it must rely on one of the other stress responses such as the response enacted by the autonomic nervous system. The autonomic nervous system is responsible for the “fight or flight” response (Cannon, 1927). This systems responses can alter the functions of the cardiovascular system, exocrine glands, as well as the digestive system. This results in changes such as altered heart rate and digestive function, but these symptoms are a short-lived reaction to the stressor.

However, the effects of the stress response from the hypothalamic pituitary neuroendocrine system last much longer and can have long term detrimental effects on immunity, stereotypical behaviors, metabolism, and reproduction. During times of long-term stress, the animal enters into a stage called prepathology and pathology. The first stage of this process, occurs when the animal has experienced a level of stress that has allowed it to become immunocompromised. If the stress persists, the animal will enter into the pathology stage where it can contract disease, experience growth retardation, cause reproductive failure, or exhibit stereotypical behavior such as tail biting (Moberg, 1985). During this stage, the animal’s biological resources are redistributed to help the animal adjust to the stressor. This means there

are less resources for the animals' normal functions, such as growth or reproduction, and the animal enters a period of distress.

Distress can be brought about by both short term and long-term stress, as well as subclinical stress. Subclinical stress can be defined as when an animal is experiencing a mild stressor that does not affect the animal in itself, however, brings the animal's immune system to the point that if it is subjected to further stress, it will affect the animals normal bodily functions. Farm animals are particularly vulnerable to the effects of subclinical stress due to the unnatural environment they live in, along with the various stressors they are subjected to by typical farm operations (Moberg & Mench, 2000).

Pigs can react to stress in several ways. One of the most common ways they choose to alleviate stress is by exhibiting stereotypical behaviors. These are behaviors that often are repetitive and have no obvious goal, such as vacuum chewing. Other behaviors include excessive elimination, diarrhea, increased activity level, hyperventilation, increased vocalization, blotchy skin, refusal to move, and tail/ear biting. Overall, stress in swine can affect the industry in five main categories, performance, reproduction, meat quality, immune function, and behavior (Martínez-Miró et al., 2016). Because stress can have such a negative effect on animal wellbeing, as well as the industry, it is important to know how any new practice affects the pigs mentally and physically. Especially if the new practice is known to have mood altering effects.

TEMPERMENT TESTING

One way to assess the impact of an experimental procedure is to perform tests to assess the temperament of the effected animals. Because animals cannot tell us how they are feeling, we must look at behavioral, physical, and biological indicators to give us some insight into the animal's state of mind. This is not only an important indicator of animal welfare, but also serves as an insight into how this new practice could impact production. Animals who are subjected to stressful farming practices can undergo mental hardship that can go on to impact their performance. Therefore, temperament testing is a valuable tool that can be utilized to help researchers assess an animal's state of mind.

Temperament is described as behavior of an individual that remains static over long periods of time and remains consistent in various situations. It is also known as the animal's nature, which includes how it reacts to stimulation, as well as the strength, and speed of that response (Réale et al., 2007). A pig's temperament is commonly measured by the strength and speed of its reaction to different stimuli and remains constant across breeds. One of the main research questions this study aims to address, is how will ingesting hempseed cake affect the behavior of the pigs, and how will it affect production. Because behavior is an indicator of mental condition, behavior is used to make judgements on the pig's mental welfare. To address these issues, the following tests are implemented into the project and will be used to assess temperament, handling, and behavior.

Scale Test

One form of temperament test is the scale test. During this test, pigs are weighed, and various scores are assigned based on the pig's performance. This test is comprised of three separate measurements: the load score, scale score, and vocal score. The loading score assesses the pig's temperament during the loading process on a five-point scale. Pigs with low scores indicate they showed very little signs of outward stress during the loading process, and pigs with higher scores showed a stronger inclination to exhibit behaviors indicating stress. The main indicator contributing to the pig's score is the amount of resistance the pig gives to avoid being separated from the group and going on to the scale. If the pig exhibits signs of stress such as squealing, refusal to move, or frantic activity level, these behaviors contribute to a high loading score.

The loading score evaluation begins as soon as the pig is singled out for weighing and ends once they enter the scale and the door is closed. Once the loading score is assessed, the scale scoring process begins. This is also a five-point scale and is a measure of the pig's activity level during confinement. For consistency, it is important that each pig be on the scale for the same amount of time. The scale score can be assessed in person, but to avoid the observer effect, filming the weighing and scoring it later seems to be a superior option. The last score of the test is the vocalization score. Vocalizing is one outlet that pigs use to show emotions. Grunting can often indicate contentment, squealing can indicate an intense emotional state, and barking can be a sign of aggression or excitement. While the pig is on the scale, vocalizations are measured and scored on a five-point scale by an investigator standing next to the scale. The scale used for this portion of the test is modified from a study published in *Applied Animal Behavior Science* journal (Marchant et al., 2001). The intensity and repetition of the vocalizations are the basis for

the scoring during this part of the test. The scoring systems used for each test are based off of the scales used by Dr. Temple Grandin, as well as the U.S. Meat Animal Research Center (Grandin, 1993; Grandin, 1994; Voisinet et al., 1997; Holl et al., 2010; Yoder et al., 2011).

Novel Object Test

The novel object test is one way to measure how susceptible an animal is to fear when faced with a novel stimulus and gives insight into the temperament of the animal. If the animal reacts with avoidance during the test, it could indicate a higher level of caution, and therefore fear, regarding the object. If the animal produces a low latency score, this will report that the animal is less fearful and therefore has a more stable temperament that would make it less susceptible to fear. This test focuses on interaction with the object and the amount of movement during the test. It starts as soon as the animal enters the distraction free pen. Once inside the pen, the animal is given a time period to adjust to its surroundings, and then a novel object is lowered by rope into the pen and observations are made over a set amount of time (Forkman et al., 2000; Kooij et al., 2002; Spake et al., 2012).

Voluntary Human Approach Test

The voluntary human approach test is a common assessment used to determine an animal's fear of humans. This test has been validated by Welfare Quality, Animal Welfare Indicators, as well as Horse Welfare Assessment Protocol (AWIN, 2015). It involves introducing an animal into a distraction free pen with a stationary novel human and measuring the animal's behavior (Forkman et al., 2007). During the test, the human avoids making eye contact with the test subject and does not initiate contact with the animal. Latency time for the animal to come in contact with the human is the main measurement during this test, however, other data such as

urination, defecation, movement within the pen, and vocalization can also be valuable to record during this test because they can also be indicators of the fear response (Norscia et al., 2021). Research has shown that swine that are fearful of humans, yield lower reproductive rates, which contributes to a 20% variation in reproductive reports (Hemsworth et al., 1989; Moberg & Mench, 2000). Pigs exhibiting an acute stress response to the human can exhibit behavior such as avoidance of the novel human, but in circumstances where the pig cannot avoid the human, such as would be the case in industrial farms, the pig may go into a chronic stress response which can lead to physiological repercussions as a result of extensive stimulation of the hypothalamic pituitary adrenal axis (HPA axis). This causes disruption of endocrine function and protein metabolism, and can eventually lead to compromised immunity, lower reproductive rates, and a decrease in the feed conversion rate (Klasing, 1985; Moberg, 1985; Clarke et al, 1992; Moberg & Mench, 2000).

Stereotypical Behavior

Stereotypical behaviors are behaviors that an animal does repetitively that seemingly serve no purpose. Examples of these behaviors in pigs include vacuum chewing, rooting, bar biting, and belly nosing. Belly nosing is often seen in prematurely weaned pigs and comes into full effect up to one week after weaning. Pigs exhibiting this behavior can spend up to 25 minutes per day engaging in it and can injure other pigs in the process (Keeling & Gonyou, 2001). Belly nosers tend to have a decreased feed intake and therefore reach market weight later than non-belly nosers (Widowski et al., 2008). However, research shows that utilizing bowl waters rather than nipple drinkers, as well as feeding a liquid diet, can reduce the instance of belly nosing (Orgeur et al., 2003; Torrey & Widowski, 2004).

Although stereotypical behaviors were first recognized in laboratory and zoo animals, it is widely recognized that performing these actions is one way that pigs can indicate stress, as well as their level of feed motivation (Moberg & Mench, 2000). Researchers have made this connection because research shows that pigs performing stereotypical behaviors have a diminished level of cortisol, a commonly used biomarker used to indict stress in swine (Martínez-Miró et al., 2016). Due to the presence of this biomarker, it is thought that performing stereotypical behaviors helps the animal to cope with stressors. Studies have linked these behaviors to lack of stimulation in their housing system, age, presence of hunger, inability to express natural behaviors, and seeing other animals expressing these behaviors. Additionally, researchers have discovered that confinement, feed availability, and the absence or presence of straw effect, the frequency with which stereotypical behaviors are exhibited (Vestergaard & Hansen, 1984; Rushen, 1985; Dantzer, 1986; Appleby & Lawrence, 1987; Jensen, 1988; Von Borell & Hurnik, 1991).

One study by the University of Minnesota found that 77% of stereotypical behaviors occurred before feeding. Studies have also indicated a rise in behaviors such as vacuum chewing and bar biting two hours after a feeding event (Rushen, 1985; Jensen, 1988; Arellano et al., 1992). Stereotypical behaviors can also have a negative effect on the offspring of the animal exhibiting them. The “thrifty phenotype hypothesis” states that the environment that a pregnant individual lives in, will change the development of the offspring so that they can be better suited for that specific environment (Hales & Barker, 2001). Characteristics such as response to stress, emotional intelligence, and mental perception are thought to be influenced by the pregnant mother’s environment during gestation (Poletto et al., 2006; Weinstock, 2008; Rutherford et al., 2014). A study by the University of Sao Paulo studied the effect of sow stereotypical behavior

expression and the behaviors of their offspring and found evidence to support this theory (Tatemoto et al., 2020).

Maintenance Behaviors Indicating Welfare Status

Basic behaviors that animals perform, such as sleeping, being active, eating, and drinking, provide the animal the bare minimum requirements to stay alive. Therefore, when a disruption in the performance of these behaviors occurs, it is an indicator that the animal is not physically and/or mentally sound. For example, 15-25% of a pig's day is spent being active, while the remainder of the time is spent resting. While resting, if they are in a thermal comfort zone, they typically lay laterally with half the animals making physical contact (Eckel et al., 2003). When a pen of pigs is huddled together, this can indicate they are too cold, and when the pigs are all spread out, this indicates they are too hot. Because pigs do spend much of their time resting, if they experience an increase in the amount of time they are inactive, this is a sign that something is wrong.

One very important maintenance behavior is eating and drinking. Pigs will spend between 5-10% of their time engaging in this behavior. Pigs in the nursery may engage in 20-25 feeding events per day, and pigs in the finishing barn may engage in only 10-15 feeding events. These events occur more often in the morning and in the afternoon (Hyun & Ellis, 2001; Hyun & Ellis, 2002). While most feeding events happen during the day, event occurrences will become more frequent at night if the competition for feed is elevated, therefore, altering the pigs eating pattern. A pig's eating pattern is also influenced by the eating pattern of other pigs. This is why pigs will typically eat at the same time, unless there is not sufficient feeder space. Not only are pigs influenced by other pigs eating patterns, but they also take a "follow the leader" style when

it comes to a new diet or deciding which feeder they favor the most (Hsia & Wood-Gush, 1984; Nicol & Pope, 1994).

When the pig is in a comfortable thermal environment, they will typically engage in drinking within the first 10 minutes after each feeding event. Therefore, the number of times they drink per day, is often correlated with the number of times they eat per day (Keeling, 2001). Finisher pigs engage in drinking behavior for 15-30 seconds at a time and spend on average up to 30 minutes per day drinking water. However, these numbers can be influenced by thermal stress and sickness, therefore, water intake can serve as an indicator of wellbeing. The elimination behaviors of pigs are also maintenance behaviors that can be used to decide welfare status as well. Swine like to eliminate in thermoneutral, damp, and low traffic areas, most commonly the corner of the pen. In many swine barns, pens have open slates in the back of the pen where pigs typically choose to dung, especially along the edge of a neighboring pen. It is thought that they like to eliminate in this area because of the air flow created by the pit, or because of their territorial instincts. An increase in elimination behaviors can be seen when animals are sick, afraid, stressed, heat stressed, or in overcrowded pens (Hacker et al., 1994; Huynh et al., 2005; Li, 2014)

GLUCOSE AND LACTOSE LEVELS

When an animal encounters a stressful event, the body goes through a series of physiological responses to help the animal handle the situation they are facing. As a part of this response, hormone, enzyme, electrolyte, and metabolite levels fluctuate in the blood and can be measured to give researchers an indication of an animal's mental and physical state. Not only do these measurements provide an indication of animal welfare during production, and pre or post slaughter, but they can also give producers an indication of how their farming methods impact their profitability (Choe et al., 2015; Čobanović, et al., 2017; Somnavilla et al., 2017).

Two key stress metabolites that can serve as an indicator of stress levels and meat quality, are glucose and lactate. When these levels are found to be elevated, not only does it indicate a high level of stress, but it also serves as an indicator of potential pale, soft, and exudative (PSE) presence in the product. These metabolites are released by rapid muscle and hepatic glycogenolysis as a response to the activation of the sympathetic adrenal medullary axis where adrenaline and noradrenaline are also released as a part of the acute stress response (Stewart et al., 2018; Loudon et al., 2019). When an animal encounters stress, the hypothalamic pituitary adrenocortical axis and the sympathetic nervous system are both activated and cortisol is released into the blood stream which then activates gluconeogenesis. Gluconeogenesis will cause the glucose levels in the blood to rise. Additionally, during the stressful event, ruminants and non-ruminants will utilize anaerobic glycolysis to supply themselves with energy, which in turn will raise the level of lactate in the blood (Warriss, 2010; Foury et al., 2011; Choe & Kim, 2014; Choe et al., 2015).

Several things can influence the circulation of glucose and lactate in the blood profile of an animal. Some of these include animal handling practices, barn conditions, and pen mate status. One study focusing on the presence of stress biomarkers correlation to the season with which slaughter takes place, found that lactate levels were higher in the summer compared to other seasons. Genetics play a role in glucose/lactate presence in the blood as well. Pigs have been bred to improve product yield and this has resulted in an increased amount of glycogen in the muscle fibers. Pigs that have the stress carrier gene (Nn genotype), have up to three times higher glycogen than pigs without it. This can also influence the amount of glucose and lactate found in the animal's blood (Kocwin-Podsiadla et al., 1995; Foury et al., 2007; Edwards et al., 2010; Čobanović et al., 2019). The method in which the lactate and glucose are measured can also play a role in the accuracy of the results. For example, when tested as whole blood, these measurements tend to be lower than the true measurement (Rocha et al., 2015; Čobanović et al., 2020). While welfare can be assessed biologically and visually via observing behaviors performed by the animals, the state of the animal's body can also be an indicator of adequate or inadequate welfare.

CARCASS TRAITS

Scoring lesions on a carcass is one-way researchers can judge animal welfare as well as potential product damage. Carcass damage can often come in the form of chewed ears, tail biting, shoulder/leg sores, and scratches or bruises along the body. Lesions indicate physical events that the animal has endured prior to slaughter and can result from electric prod use, abuse, confinement, and fighting. Several factors influence fighting behaviors in pigs. For example, in colder temperatures, pigs huddle together to create warmth. This creates a situation in which pigs are competing for space within the huddle, which can cause pigs to fight. Additionally, in warmer temperatures pigs like to lay on cool surfaces to redistribute heat from themselves and onto another surface. This thermoregulation method is called conduction. Pigs typically use the floor in their pen to employ this method but when they are not able to do this, they become short tempered and are more likely to engage in fighting with other pigs (Gosálvez et al., 2006; Olczak & Nowicki, 2015; Čobanović et al., 2016). Therefore, time of year plays a large role in lesion prevalence at slaughter. The hotter months typically yield the highest amount of carcass lesions. However, this rate is exacerbated further when there are many gilts in the herd because the hormones involved in sexual maturity can contribute to an animal's temperament (Di Martino et al., 2018).

The amount of time a pig is fasted prior to slaughter can also contribute to the prevalence of fighting. Pigs held overnight in a plant prior slaughter were found to be at a higher risk of fighting because the longer they fasted, the more temperamental they became (Nanni Costa et al., 2002; Sterten et al., 2009). Tail biting is one form of redirected rooting behavior that can cause

significant damage because of the potential of injuring the spinal cord and appears to be linked to stress level (Zupan et al., 2012). One study found that this behavior could have a link to genetics and that Landrace breeds were not only more likely to perform this behavior but were also more likely to pass it to their offspring as well (Breuer et al., 2005; Munsterhjelm et al., 2013).

CHAPTER 2- THE EFFECTS OF HEMPSEED CAKE ON SWINE BEHAVIOR

INTRODUCTION

Hemp is a plant that is grown and used for many purposes. Its scientific name is *Cannabis sativa* L. The main products of the hemp plant are fiber and the oil that is pressed from the seed. It has been used for consumption, animal feed, medical purposes, wood preservation, soap, ink, skin moisturizer, and detergents (Oomah et al., 2001). Hemp and marijuana are often thought of as the same product, but they actually have several key differences. While they both derive from the same plant, *Cannabis sativa*, they differ in fiber content, amount of Tetrahydrocannabinol (THC) and Cannabidiolic Acid (CBDA) they contain, and the use of their seeds for oil (West, 1998). In humans, the oil from the hempseed is thought to relieve symptoms of diabetes, eczema, arthritis, pain, balding, post menstrual symptoms, aid in fetal development, improve cancer symptoms by decreasing inflammation, improve heart health, reduce depression/anxiety, improve memory, boost immunity, and improve digestion and weight loss (Hall, 2019). One study that included 6,462 subjects found evidence that the use of cannabinoids relieved symptoms of spasticity and long-lasting pain. They also found some evidence that suggested cannabinoids could possibly be used to relieve symptoms of cancer treatment, weight changes in human immunodeficiency virus (HIV) treatment, Tourette's, and conditions involving sleep disturbance. However, symptoms such as vomiting, fatigue, dry mouth, hallucinations, diarrhea, confusion, and difficulty keeping balance, have all been reported side effects of

cannabinoid use (Whiting, 2015). The use of hemp has not only penetrated the market for human use, but it has also found its way into the pet market for use in the form of treats, oil, and pills. Cat and dog owners who distribute this product to their animals, report that it helps relieve arthritis symptoms, stabilize mood, reduce seizure and irritable bowel syndrome (IBS) episodes, relieve nausea, pain, and stabilize appetite (Noland, 2013). As hemp use becomes more and more popular, it is not surprising that hemp use has also made its way into agriculture.

When being used as a feed additive, hemp is often made into a structure called a hempseed cake. The hempseed cake is comprised of crude protein, neutral detergent fiber, crude fiber, acid detergent fiber, crude fat, methionine, calcium, lysine, phosphorus, sodium, and selenium (USDA, 2019). Several studies have been conducted involving hemp being used as a feed additive for livestock. For example, hempseed cake inclusion in the diet of farrowing sows has been shown to have a positive effect on piglet performance (Hăbeanu et al., 2016), antioxidant enzyme activity in sows pre and post farrowing (Palade et al., 2019), and an increase of fatty acid composition in milk. This influences piglet thriftiness (Vodolazska & Lauridsen, 2020) as well as the fat profile of the pork, which can have an effect on tenderness and flavor (Mourot & Guillevic, 2015). Studies involving hemp use have been published for many livestock species such as pigs, chickens, and cows. The swine industry is in an optimal position to be able to maximize from the potential benefits that hemp has to offer, however, the Food and Drug Administration (FDA) has not currently approved hempseed cake as a livestock feed additive (Rehman et al., 2021). From a financial standpoint, one of the biggest advantages to gain by adopting this feed additive into industrial production standards could come in the form of feed expenditures. The cost of feed accounts for over two thirds of the total production costs on a swine farm. This number is influenced by the cost of diet, as well as the rate of feed conversion

(Lammers et al., 2007). Fortunately, scientists are conducting research on feed alternatives that could potentially offset these costs. Legalization of cannabis use is becoming more and more frequent. States such as Colorado, Washington, Alaska, and Oregon, have already approved recreational consumption (Ausick, 2015). As the cannabis market grows larger, so does the number of byproducts generated by the industry. If hempseed cake is identified as an acceptable protein source, then this feedstuff may be an affordable option that producers can use to offset rising feed costs.

Due to hemp being formerly regarded as a Schedule 1 agent, meaning it is likely to be abused and has no scientifically proven medical use, research has been restricted in the United States for many years. Because of this, those who are interested in utilizing it for medical purposes, veterinary medicine, or agricultural purposes, are left with a rather large knowledge gap and providers such as veterinarians and doctors do not have a reliable source of information to provide consumers with. This has led to a largely unregulated and uninformed market which has struggled to discover exactly how hemp products affect them and their animals (Nolan, 2013; Robinson, 2014). However, the industry has been able to obtain valuable knowledge about the effects of hemp in recent years due to the 2014 Farm Bill which allowed for specific agencies to perform research involving hemp (USDA, 2024). The 2018 Farm Bill stipulates that hemp is no longer classified as a controlled substance and is now regarded as a legal agricultural product that is further defined as being a separate entity from marijuana (USDA, 2018). While hemp is now legalized on the federal level, it is not legalized on the state level. Each state has its own laws regarding hemp use, but the 2018 Farm Bill still allows for the transport of hemp through any state regardless of local laws (DuBois & Bridges, 2020). With the increase in legalization and interest in the hemp market, combined with the growing needs of the swine industry, it is

more important than ever to conduct meaningful research to discover how hemp impacts the wellbeing and behavior of pigs. This type of research is pivotal to the industry so that it may evolve in a welfare friendly and economically sustainable fashion.

The study hypothesis was that the use of hempseed cake as a protein source in swine diets would modify animal behavior and post-mortem responses. Therefore, the objective was to compare the animal behavior responses including temperament tests, response to human approach, novel object test, and post-mortem glucose and lactate between the experimental group fed the hempseed cake diet versus the control group fed a traditional soybean diet. For this experiment, behavior was used as an indicator of animal welfare.

MATERIALS AND METHODS

Study farm and animals

Forty-three piglets were bred from a Yorkshire X Landrace cross (DNA genetics line 241 F1) sow and a purebred Duroc boar (DNA genetics line 600). Originally the experimental group and control group both had 22 pigs, however one was removed due to illness. Conception took place following artificial insemination from pooled semen doses, containing five sires per dose. At nursery age, piglets were moved from the farrowing barn to an open barn where pigs from outside of the project were also housed. The barns were located in College Station, Texas and study pigs were housed there during the months of June, July, August, and September, when temperatures were often very high during the day. Neither misters, nor curtains were employed in the barns. The pigs used in this project were housed in a total of four 9.68 m by 2.48 m pens. The front of the pens were made up of solid concrete flooring and the back 6.10 m of the pens were comprised of slatted concrete flooring. Pigs fed the same diet were housed in pens next to each other, with the opposing diet group housed across the alley. The control group containing 22 piglets was fed a diet of traditional soybean meal, while the experimental group containing 21 piglets were fed a diet that supplemented hempseed cake as the main protein source. Each ration was made on an onsite feed mill with a total of 907.18 kg of feed mixed per ration, per phase. Ractopamine was not included in the feed rations. Feed was distributed ad lib using Platinum Series 300 Hog Slat feeders located at the Texas A&M Swine Center. The feeders were single sided with four feeding stations. They measured 1.27 m in length, 0.91 m tall, 0.39 m wide, and had 0.14 m of feeder depth. The feeders weighed 42.18 kg and had a capacity to hold 215.46 kg of feed. The feed rations for each group were changed every 30 days to reflect typical industry

protocols. Additionally, every 30 days the pigs were weighed and behaviors scored using Table 3. During phase two, the piglets were evaluated during a novel object test and a voluntary human approach test. After phase three was completed, the pigs were slaughtered over a one week period. The pigs were evaluated post mortem to check glucose and lactate levels in the blood. The remaining feed was also weighed to determine the feed usage for the control and experimental groups. The classification frequencies of the study pigs for the loading and vocalization scores over the observation times were calculated and placed in Table 3.

Study design

The objective was to compare the animal behavior responses including three temperament tests performed monthly, response to human approach, novel object test, and post-mortem glucose and lactate measurements between the experimental group fed the hempseed cake diet versus the control group fed a traditional soybean diet. This was a randomized controlled study that used behavior as an indicator of animal welfare. Forty-three piglets were bred from a Yorkshire X Landrace cross (DNA genetics line 241 F1) sow and a purebred Duroc boar (DNA genetics line 600). The pigs chosen for the study were randomly allocated into two groups (TRT = hemp; CON= soybean) and distributed in four pens (TRT pens, n = 2; CON pens, n = 2). The study was conducted in three 30 day phases (94 days in total). Each ration was formulated to be isocaloric and at the beginning of each phase the protein source of the control group and the experimental group decreased to mimic swine industry standards. Phase one began at 16 weeks of age, phase 2 began at 20 weeks of age, and phase 3 began at 26 weeks of age

Figure 1. Timeline of events

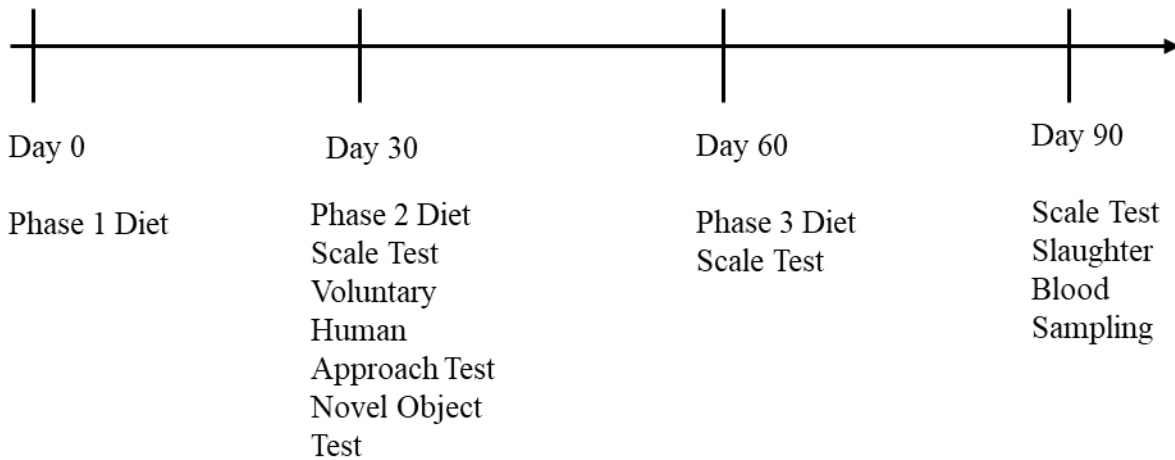


Figure 1: The diets formulated in this study were created to follow the industry standard of decreasing the protein composition of the feed at each phase. In phase 1, the hempseed cake composition of the feed was 41.17%, in phase 2 it was 33.63%, and in phase 3 it was 25.18%. In phase 1, the soybean composition of the feed was 31.04%, in phase 2 it was 21.09%, and in phase 3 it was 14.19%.

Table 1. Nutritional analysis of diets by phase, % dry matter and decrease of protein content

	Protein	Dry Matter	Moisture	Crude Protein	ADF	aNDF	Fat
Phase 1							
Soybean Meal	31.04	90.4	9.6	20.4	3.4	8	4.4
Hempseed Meal	41.17	88.5	11.4	18.7	16	19.9	9.7
Phase 2							
Soybean Meal	21.09	90.8	9.1	16.9	2.5	8.3	3.3
Hempseed Meal	33.63	89.1	10.8	16.6	11.8	20.1	9.2
Phase 3							
Soybean Meal	14.19	90.3	9.6	15	2.6	12.4	2.1
Hempseed Meal	25.18	88.7	11.2	15.1	9.4	16.4	7.6

Modified from Kirkpatrick Kemp et al., 2022.

Table 1: Phase 1 represents the diet that was fed during days 0-30. Phase 2 represents the diet that was fed from day 30 to day 60. Phase 3 represents the diet that was fed from day 60 to day 90.

Outcome variables.

The outcome variables of this study were measured to assess the effects of the experimental feed utilizing hemp as a protein source on the welfare of the pigs and post-mortem stress levels. The variables considered in this study were temperament tests (loading and vocalization scores), human approach test, a novel object test, and glucose and lactate measurements taken post-mortem from whole blood.

Temperament tests

Four pens containing 43 pigs were weighed once a month from the beginning of the trial in which temperament tests were performed. One pen would be herded down the alley way by three herdsman with red sorting boards to a pen further down the barn that was adjacent to a Paul scale at the Texas A&M Swine Center (College Station, Texas). The scale was in the alley way,

perpendicular to the walkway. Once all pigs were loaded into the pen area, the three herdsman would use the sorting boards to separate one pig from the herd and move it towards the entrance of the Paul Scale. Once loaded into the scale, the gate to the scale would be closed and the pig would be facing white wooden boards meant to eliminate visual distraction from pigs across the alley way. During the loading process, a researcher stood in the right corner of the adjoining pen closest to the alley way and visually evaluated the temperament of each pig and assigned loading and vocalization scores according to the scale described in Table 2. Once the pig was loaded onto the scale, the researcher would start a timer and stand directly beside the scale to evaluate behavior, measure body weight, and record vocalizations during a 30 second time period. After 30 seconds, this pig would be released from the scale and the pig's behavior as they exited the scale was recorded by a camera placed overhead and later evaluated and scored according to the specifications in Table 2. Once the pig exited the pen, it would go to the left and walk through a gate where it would walk down the alley way and stay there until weighing of the entire pen was complete. Once weighing was complete, the herdsman would use the sorting boards to herd the pigs back into the home pen and repeat the process with the next pen until all pigs were weighed and evaluated.

Table 2. Temperament tests assessed during study pig weighing days

Score levels	Loading Score	Vocalization Score
1	Required gentle guiding with a sorting board	No vocalization
2	Turned around upon approaching the scale and/or required three or less touches by herdsman or pushes from sorting board	Admitted sporadic low toned grunts
3	Turned around upon approaching the scale and/or required three or more touches by herdsman or pushes from the sorting board	Admitted repetitive low toned grunts
4	Turned around upon approaching the scale, required more than three touches by herdsman and/or pushes from the sorting board, and tried to escape	Admitted sporadic squealing
5	Turned around upon approaching the scale, required more than three touches by herdsman and/or pushes from the sorting board, loudly vocalized, and made many attempts at escape	Admitted repetitive squealing

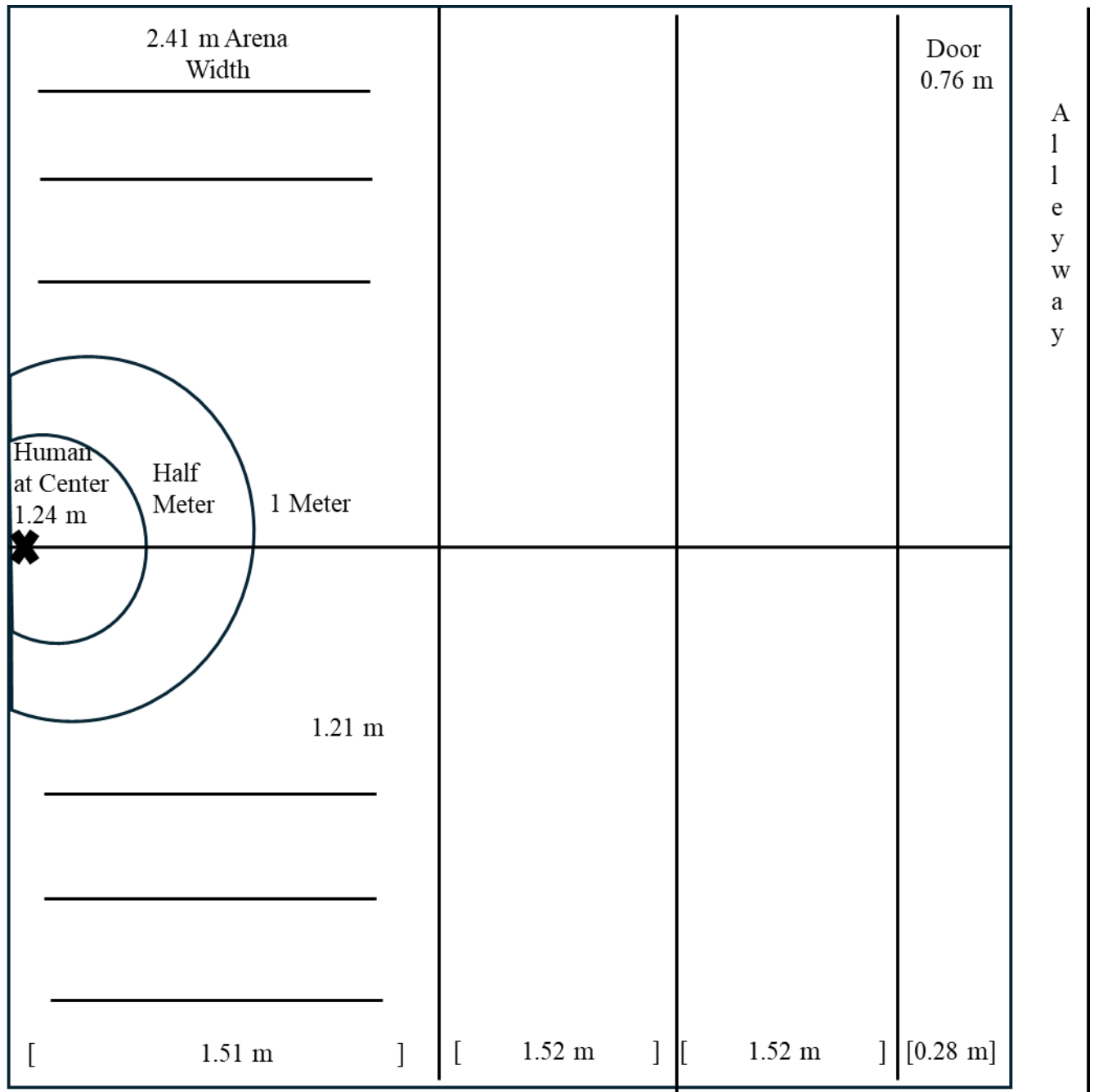
Modified from Grandin, 1992; Grandin, 1994; Voisinet et al., 1997; Marchant et al., 2000; Holl et al., 2010; Yoder et al., 2010.

Voluntary Human Approach Test

A voluntary human approach test was performed at the end of phase one, day 30. A pig was separated from its pen mates and herded out of the home pen by herdsman with red sorting boards and led down an alley way to the testing arena where an unfamiliar human was standing in the middle section of the far end of the arena with their back against the pen wearing a bright orange t-shirt over blue coveralls and tall farm boots. The arena, (Figure 2), was comprised of square slats in the floor, a one-meter half circle around the human, as well as a half meter half

circle around the human. The markings were made with black spray paint and the floor was wet. The arena was lined with white wooden boards to minimize distractions. Two cameras were located above the pen and an investigator was in the barn, watching the pig's behavior over a monitor. Once the pig entered the pen and the pen door closed, the investigator began a timer and observed the behavior over a five-minute time period. During the observation period, the investigator recorded latency to enter the one-meter mark, latency to enter the half meter mark, latency to touch the human, number of contact bouts, number of grid crossings, and number of urinations and defecations. Once five minutes had passed, the investigator signaled the herdsman to remove the pig from the arena, take it to a third location away from the home pen and arena, and bring in another pig. The pen and the human's boots were hosed down between tests to avoid a scent confound between trials. The researcher was blind to treatment during assessments (Pairis et al., 2009).

Figure 2. Voluntary human approach testing arena diagram.



White Wooden Boards Lining Border 1.24 m Tall

Figure 2: The testing arena was comprised of concrete slatted flooring that created a grid in the floor.

Novel Object Test

The novel object test was performed at the end of phase one at day 30. A pig was separated from its pen mates and herded out of the home pen by herdsmen with red sorting boards and led down an alley way to the testing arena. The arena, as shown in Figure 3, was lined with white wooden boards to minimize distractions and was wet with slatted flooring that was used as a grid. A camera was placed above the pen and was transmitted to a monitor located in the barn, where a researcher was observing and recording behavior. Once the pig was led into the pen and the door was closed, a one-minute timer began and the pig was left to acclimate itself during this time. The grid crossings were recorded via video monitoring. After one-minute passed, a 73.66 cm by 38.1 cm orange traffic cone, weighing 10.7 kg was dropped from the ceiling via a pulley system, and behavior was observed for four minutes. Latency to touch the novel object, number of contact bouts, number of grid crossings, and number of urinations and defecations were recorded during this time. After the observation period ended, the investigator signaled the herdsmen to remove the pig from the arena, take it to a third location away from the arena and home pen, and bring in another pig. Before a new pig entered, the arena and traffic cone were hosed down with water to avoid a scent confound between trials. The researcher recording behavior was blind to treatment during assessments.

Figure 3. Novel object testing field diagram.

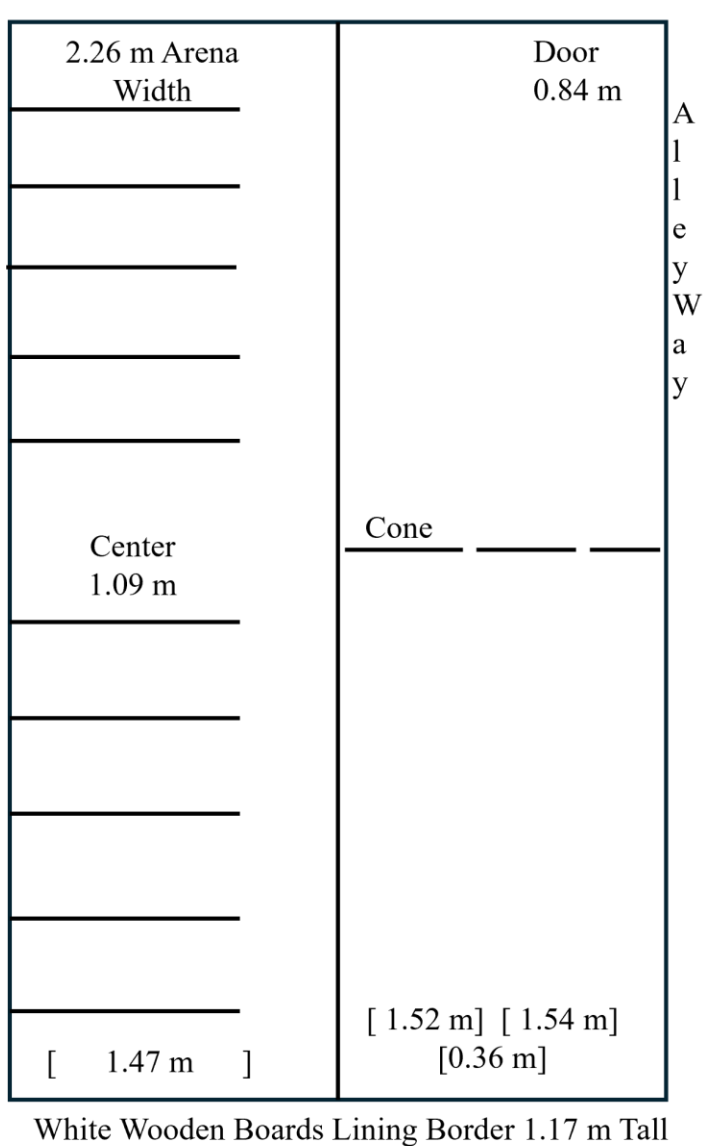


Figure 3: The testing arena was comprised of concrete slatted flooring that created a grid in the floor.

Glucose and Lactate Measurements

After phase three was completed, the pigs were slaughtered over a one-week period and glucose and lactate levels were evaluated postmortem from blood. The remaining feed was also weighed

to determine the feed usage for the control and experimental groups. The workstation was equipped with the glucose meter and strips, lactate meter and strips, solo cups, needles, syringes, tubes, gloves, and sharps container. Once the pig coming out of the lairage was identified, a researcher signaled the pigs identifier and after the animal was stunned and the heart stick was made, an investigator collected the blood in a solo cup and brought it to the work station. A timer started to ensure the blood was tested promptly, and in an attempt to avoid coagulation. Then a 3 mL syringe with needle was injected into the blood sample and 2 mL was withdrawn and placed into a EDTA tube. The tube was inverted several times and a new syringe with needle was inserted into the tube and a small amount was drawn into the syringe. The lactate meter was prepared by turning it on and inserting a strip. The syringe was inverted, and a small amount of blood was placed onto the strip. The reading was recorded on the score sheet. The glucose meter was prepared by turning it on and inserting a strip. The syringe was inverted, and a small amount of blood was placed onto the end of the strip and the reading was recorded.

Statistical analysis

All statistical analyses were performed in SAS (9.4 SAS, NC). Descriptive statistics including mean and standard deviation of the outcome variables were calculated using PROC MEANS for continuous variables and frequency analysis for categorical variables (PROC FREQ). To determine the effect of the dietary intervention of the outcome variables we used mixed models (PROC MIXED) considering treatment allocation and fixed effect and pig ID as a random effect. Least square means (LSM) and standard error of the mean (SEM) are reported. Statistical significance was determined at p-values <0.05.

RESULTS

This study aimed to compare the behavioral responses of a group of experimental pigs fed a diet supplemented with hempseed cake to a control group fed a traditional soybean diet. To measure these responses, they were subjected to three temperament tests performed monthly, a novel object test, a voluntary human approach test, and a glucose and lactate test performed post mortem. This study took place at a finishing barn in College Station, Texas and utilized 43 pigs. The pigs were a cross between a Yorkshire X Landrace sow and a purebred Duroc boar, and were housed in a total of four 9.68 m by 2.48 m pens. The front of the pens were made up of solid concrete flooring and the back 6.10 m of the pens were comprised of slatted concrete flooring.

Table 4 shows descriptive statistics (mean and standard deviation) of the study pigs enrolled in this study considering temperament test (loading and vocalization scores) measured at days 30, 60, and 90 of the study. Loading scores initially increased and then decreased, potentially as a result of the pigs becoming accustomed to the scale. However, the means of the vocalization scores steadily declined. Additionally, we calculated the classification frequencies of the study pigs for the loading and vocalization scores over the observation times (Table 3). Except for the loading score for day 60, the data shows an increase in the frequencies of a loading and vocalization score of 1.

Effects of a hempseed cake diet on pig behavior

Based on the statistical analysis performed in this study, the inclusion of hempseed cake in the diet did not produce many significant findings. Table 3 shows that outside of the significant findings on the day 60 loading score, the data in this table follows a similar pattern as shown in Table 4. With each test performed, the percentage of occurrence of the lower scores increases. The only differences in the loading scores were observed on day 60 (CON: 2.0 ± 0.16 vs. TRT 1.46 ± 0.16 , $P = 0.01$). On the other hand, no differences between treatments were found for the vocalization scores.

Glucose and Lactate concentration (Table 5) as well as behavior during the Novel Object Test (Table 6) were similar between the two treatments. During the Human Approach Test (Table 7), TRT pigs took longer to approach a human within 1 m ($P = 0.04$) and made fewer grid crossings during the test ($P = 0.02$).

Table 3. Frequency Analysis. Loading scores and vocalization scores at day 30, day 60, and day 90 paired with the percentage of occurrence of the score in the treatment and control group and the Chi-sq P-value.

Loading Score					Vocalization Score						
Day 30	Treatment (n)	%	Control (n)	%	Chi-sq P-value	Day 30	Treatment (n)	%	Control (n)	%	Chi-sq P-value
1	10	23.26	12	27.91	0.58	1	12	27.91	13	30.23	0.58
2	8	18.6	9	20.93		2	8	18.6	9	20.93	
3	2	4.65	0	0		3	1	2.33	0	0	
4	1	2.33	1	2.33		4	-	-	-	-	
5	-	-	-	-		5	-	-	-	-	
Day 60						Day 60					
1	5	11.9	14	33.33	0.03	1	14	33.33	19	45.27	0.06
2	12	28.57	5	11.9		2	4	16.67	2	4.76	
3	3	7.14	2	4.76		3	-	-	-	-	
4	1	2.28	0	0		4	-	-	-	-	
5	-	-	-	-		5	-	-	-	-	
Day 90						Day 90					
1	11	26.83	17	41.46	0.17	1	17	41.46	20	48.78	0.26
2	8	19.51	3	7.32		2	3	7.32	1	2.11	
3	1	2.44	1	2.44		3	-	-	-	-	
4	-	-	-	-		4	-	-	-	-	
5	-	-	-	-		5	-	-	-	-	

Table 4. Least square means and standard error of loading and vocalization scores of pigs fed a hemp diet (treatment) compared a soybean diet (control).

Variable	Treatment	SEM	Control	SEM	P-value
Loading score					
Day 30	1.71	0.17	1.55	0.17	0.48
Day 60	2.00	0.16	1.43	0.16	0.01
Day 90	1.50	0.13	1.24	0.13	0.15
Vocalization score					
Day 30	1.58	0.16	1.52	0.16	0.71
Day 60	1.33	0.09	1.10	0.09	0.06
Day 90	1.15	0.06	1.04	0.07	0.28

Table 5. Glucose and Lactate Measurements. Comparison of the lactate and glucose measurements between the control group and treatment group using the standard error mean and the P-value.

Variable	Treatment	SEM	Control	SEM	P-value
Lactate (mmol/L)	4.12	0.52	3.29	0.51	0.26
Glucose (mg/dL)	114.25	6.05	120.95	5.91	0.43

Table 6. Novel Object Test. Six variables measured during the novel object test comparing the treatment group vs the control group using the standard error mean and the P-value.

Variable	Treatment	SEM	Control	SEM	P-value
Time to contact (s)	170.67	17.87	145.67	17.87	0.33
Contact time (s)	82.48	21.28	57.76	21.28	0.42
Number of contacts	1.52	0.23	1.86	0.23	0.31
Grid pre-object	5.29	0.49	4.14	0.49	0.1
Grid post-object	15.52	2.00	17.14	2.00	0.57
Defecations	0.52	0.16	0.14	0.16	0.1

Table 7. Human Approach Test. Behavior of pigs fed either the treatment or control diet during a human approach test that was conducted X days after treatment implementation.

Variable	Treatment	SEM	Control	SEM	P-value
Latency to Enter 1M (s)	55.79	9.06	28.79	9.06	0.04
Duration in 1M (s)	50.21	7.91	57.89	7.91	0.49
Latency to Enter 1/2M (s)	134.84	24.41	116.84	24.41	0.6
Duration 1/2M (s)	97.89	28.74	127.68	28.74	0.38
Latency to First Contact (s)	123.79	22.32	127.58	22.32	0.9
Contact Duration (s)	122.95	32.50	136.53	32.50	0.77
Bouts	1.05	0.21	0.79	0.81	0.38
Grid Crossings	14.47	1.64	20.16	1.64	0.02
Defecations	0.11	0.13	0.37	0.13	0.17

DISCUSSION

Overall, this pilot study did not produce many significant findings to indicate that the inclusion of hempseed cake in the diet of pigs would lead to significant changes in a pig's behavior during a novel object test, human approach test, glucose/lactate measurements, or loading/vocalization tests in a chute. One finding to note is the number of grid crossings during the human approach test. The experimental group had an average of 14.47 grid crossings vs the control group who had an average of 20.16 grid crossings and a p-value of 0.02. This indicates that the experimental group had reduced activity compared to the control group, and that this finding is significant due to the low p-value. This result aligns with current findings in cats, dogs, and humans that hemp can have a calming, or antianxiety effect (Kogan et al., 2016; Hall, 2019). While this finding was significant, overall the feed did not affect the behavior of the pigs. While it is possible that some effects were missed during this study for various reasons, the minimal impacts observed in this study suggest that hemp could be used as a protein substitute for soybean without negatively affecting the welfare of pigs. As the agricultural industry looks for more affordable sources of protein, research studies like this can be an important contribution to the field by producing evidence for or against the inclusion of novel feed ingredients by studying their potential effects on behavior. Glucose and lactate are two measurements that can be indicators of an animal's physical and mental state, as well as stress level. These metabolites can be influenced by diet (Choe et al., 2015; Čobanović, et al., 2017; Somnavilla et al., 2017). Animal welfare can be assessed by studying animal behavior and several studies have shown that nutrition can play a direct role on animal behavior. For example, a diet low in protein has been shown to lead to an increase in biting which has been shown to have a link with lower feed to gain ratios (Beattie et al., 2005; Meer et al., 2017). Protein deficiency in pigs has been shown to lead to pigs being

drawn to the taste of blood, as well as rooting (Fraser et al., 1991; Jensen et al., 1993). These behaviors have been shown to lead to an increase in instances of ear biting, which negatively impacts animal welfare (Hewett et al., 2025). While industry standards (such as those that this project followed) account for the pig's nutritional needs, especially protein at each phase of life, this is an example of why it is important to ensure all new feed additives are assessed for quality to ensure they are compatible with the animal's needs.

There were several limitations in this study that could have affected the results including sample size, weather, timing of the tests performed, and husbandry consistency. Also, there were various researchers involved in caring for and conducting the tests which may have led to differences in the data of the behavior tests that were conducted over several days due to a difference in animal handling. This study took place in Texas during the summer months when the weather was very hot, which could have also affected the study outcomes. The weather in Texas during the summer can often reach to over 100 degrees Fahrenheit. It is possible that the heat and/or a difference in handlers could have been responsible for the unexpected results found in day 60 of the loading score. Another possible reason could be that the pigs became accustomed to the routine of the test.

There are several possible reasons why the study may not have yielded more statistically significant results. The size of the study could have been a limiting factor, as well as the number of studies conducted. The novel object test and the human approach test were conducted at day 30; limiting the impact of the dietary treatment on behavior during the tests. If the tests had been conducted at day 90, then different results may have been observed.

CONCLUSIONS

The largest expense on a swine operation is the feed (USDA NASS, 2024), particularly high quality protein feed. Finding alternative feed components at a lesser cost can greatly impact the financial bottom line of any operation, however these novel components should be tested for safety. This study evaluated the impact of an experimental hemp diet on pig behavior and stress physiology. Few differences, including latency to approach a human and activity levels during a human-approach test, were observed during the study suggesting that the hemp diet may have a mildly sedative impact on pig behavior.

Farms need to be profitable in order to meet the financial needs of the operation, this can be significantly impacted by the quality of the product being produced, and as studies have shown, the quality of the product can be impacted by the welfare of the animals (Beattie et al., 2005; Meer et al., 2017). If the industry were able to introduce cheaper feed components without sacrificing feed quality or animal welfare, this could serve as a driving force to aid in the revitalization of this area of agriculture.

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