THESIS

STRATEGIES TO MAINTAIN MARKET ACCESS FOR PORK AND ENHANCE FUNCTIONALITY OF BEEF PROTEINS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2023

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ABSTRACT

STRATEGIES TO MAINTAIN MARKET ACCESS FOR PORK AND ENHANCE FUNCTIONALITY OF BEEF PROTEINS

African swine fever is a high-consequence foreign animal disease endemic to sub-Saharan Africa and the island of Sardinia. The U.S. is the world's third largest pork producer, and ASF introduction would severely disrupt the pork supply chain, emphasizing a need to protect market access for U.S. proteins. However, niche producers raising swine intended for exhibition may not follow stringent biosecurity protocols and livestock show circuits may promote untracked animal movement across the country, potentially exacerbating virus spread in the event of ASF incursion into the U.S. Two Qualtrics surveys designed to evaluate knowledge, understanding, and perceptions of ASF and biosecurity principles of youth swine exhibitors and adults involved in the exhibition swine industry were distributed via flyers, emails, and canvassing at livestock shows. Youth exhibitors (age 21 and under) answered questions assessing their knowledge and provided basic demographic information, including their home state and states to which they traveled for exhibitions. Adult respondents answered the same questions assessing their knowledge and provided information on their time involved in the swine industry and number of shows attended by the youth they advise (if any). Youth respondents (n = 127) lived in 14 states and exhibited in 23 states, with 35% and 28% holding membership in state and national swine organizations, respectively. When provided with a list of ASF clinical signs, 34 individuals (26.9%) correctly identified all symptoms. Twenty-nine individuals (23%) incorrectly responded that ASF has been found in the U.S., and ten (7.9%)

believed the virus cannot spread between pigs. Increased biosecurity understanding in youth exhibitors showed a significant relationship with an increase in years involved ($p<0.05$). Adult respondents ($n = 211$) had been involved in the swine industry for an average of 21 years, and the youth they advised attended 14 exhibitions in an average year. Nearly all adults (90.5%) identified direct contact with infected animals as a method of ASF transmission, while far fewer (36.39%) identified animal feed as a possible mechanism of transmission. These responses indicate highly varied knowledge of symptoms, routes of transmission, and biosecurity recommendations. Youth membership in state or national swine organizations offers a route for outreach and educational activities to enhance foreign animal disease preparedness, and adult presence at swine exhibitions allows for a wide variety of programming for all ages to better serve all levels of understanding.

Fluctuations in the beef supply chain due to COVID-19 triggered discussions on methods to fully utilize edible proteins from beef carcasses, such as collagen. One potential method is the addition of collagen powder to beef frankfurters to replace a fraction of lean grind. The inclusion of NOVAPRO® collagen powder to beef franks at three hydration levels resulted in no significant differences ($p>0.05$) in water activity, pH, or shear force values between the treatment groups. Additionally, trained sensory panelists did not discern differences between treatment or control samples when asked to rate attributes that included beef flavor intensity, seasoning intensity, springiness, and mouth coating, indicating that NOVAPRO® powder could be added to processed meat products to reduce costs without compromising product quality.

ACKNOWLEDGEMENTS

I am immensely thankful to the wonderful community around me without whom this program could not have happened.

Dr. Martin, your advocacy for your students and dedication to equity in education for all is admirable. Throughout all of this, I knew you were in my corner supporting me in whatever I wanted to do. To my committee, thank you for your guidance and advice on all things graduate school. My fellow grads, our sense of community is the best. Chloé, Colton, Emily, Zhai, Abbey, David, Tyler, Sara, Michael, Chris, Paxton, Melissa, Corley: I've learned so much from each of you and certainly would not have made it to this point without your encouragement on a daily, even hourly, basis. I will always be here for all of you.

Evan, what is there to say that I don't already tell you every day? We've come a long way from the OSU meat lab, and each day together feels like a gift. You are the joy of my life and my "why" for this entire degree. I so deeply appreciate your constant love and kindness when I felt like giving up. To my friends and family, both in Ohio and here in Colorado, I am incredibly grateful for your endless words of encouragement and unwavering belief that I could succeed in anything I chose to do. Mom & Dad, I love and appreciate you more than you know. Danika, thanks for always getting drinks when I just needed to chat. Dillon, it's easier to put our academic struggles into perspective when you are literally climbing Mt. Everest!

From the bottom of my heart, thank you. It takes a village and mine is full of wonderful people!

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1.1 INTRODUCTION TO AFRICAN SWINE FEVER

1.1.1 Characteristics: Endemicity

African swine fever (ASF), a high-consequence, reportable hemorrhagic fever caused by African swine fever virus (ASFV), results in high mortality rates in infected swine. ASFV is a double-stranded DNA virus first described in Kenya in 1921 and is the only member of the Asfarviridae family. There is no widely available vaccine against ASFV (Costard et al., 2009; Sánchez-Vizcaíno et al., 2012). Thailand and North Macedonia reported the first occurrence of ASF in January 2022 and two countries with outbreaks prior to 2007 have successfully eradicated the virus: Belgium and Czechia (OIE, 2022). It is currently not found in the United States, but is endemic to sub-Saharan Africa, Eastern Europe, and the island of Sardinia (Brown and Bevins, 2018). With endemicity in such a variety of regions and other countries exhausting all efforts to prevent the virus from crossing their borders, close contacts between humans and animals have the potential to exacerbate disease spread throughout the world and reintroduce diseases into countries where they were previously eradicated as globalization bolsters trade (Manuja et al., 2014).

Prior to recent outbreaks, de Glanville et. al. assessed the virus's potential for endemicity in Africa using spatial multi-criteria decision analysis (MCDA). Their analyses indicated the suitability for endemicity due to domestic swine transmission in much of sub-Saharan Africa, while other regions showed suitability for endemicity via wildlife reservoirs. These algorithms accounted for domestic pig population density, *Ornithodoros* tick spp. prevalence, and wild hog populations, including warthogs, bushpigs, and giant forest hogs. Domestic transmission cycles were supported by proximity to trade, tick populations, and pig density. Wildlife transmission cycles were supported by tick populations, pig density, and habitat suitability for wild hogs. Most of northern Africa is unsuitable for ASF introduction from either pathway, but the potential for spillover from wildlife should be monitored (de Glanville et al., 2014).

1.1.2 Characteristics: Transmission and Detection

In the event of an ASF outbreak in the United States, the greatest concern is transmission to the feral swine population. These pigs are escaped domestic swine, Eurasian wild boars introduced by early settlers, and hybrids of both species. The United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) estimates the feral swine population at 6 million and rapidly growing. They have been reported in 35 states, and with no natural predators and limited human control, they represent a growing threat to U.S. agriculture and livestock.

Feral swine are known reservoirs of diseases, including brucellosis, porcine reproductive and respiratory syndrome virus (PRRSV), and pseudorabies. Domestic swine raised in commercial, vertically integrated facilities have minimal contact with wildlife, as these producers follow strict biosecurity protocols. However, hobby and niche swine producers, such as those who raise animals for exhibition, often do not follow these biosecurity protocols and animals may have the opportunity to encounter wildlife. In a 2009 study, feral swine in Texas were found to cross 100- and 500-meter buffer zones around domestic swine pens, particularly those housing females. Feral-domestic contact could be mitigated by construction of appropriate fencing around swine facilities (Wyckoff et al., 2009).

ASF transmission most commonly occurs through direct contact with infected animals, tick bites, and consumption of contaminated swill. The virus remains stable in many substrates, including feed and soil. In Vietnam, with ongoing ASF outbreaks, truck cabs, employee clothing, and surfaces in high traffic areas were identified as the greatest sources of positive samples in the feed mill of a vertically integrated swine production facility. After the mill implemented extensive disinfection procedures and restricted drivers from exiting their cabs, positivity was reduced in later sampling (Gebhardt et al., 2022). Identification of such biosecurity gaps is crucial in modern pork production systems.

ASFV Georgia 2007/1 was used to study the half-life of the virus in feed ingredients over a simulated 30-day trans-Atlantic shipment. Samples were collected at 1, 8, 17, and 30 days after inoculation and ASFV was quantified by titration. The half-life in feed ingredients ranged from 9.6 to 14.2 days compared to 8.3 days in viral growth media, supporting the theory that feed ingredients increase viral stability. This study demonstrates that the transport of contaminated feed products is a viable pathway for ASF spread between countries (Stoian et al., 2019).

Treating patches of soil where deceased wild boar are found with no readily evident cause of death (e.g. hit by car or gunshot) may become important in countries where ASF is endemic, as Carlson et. al. found that the virus may survive up to two weeks depending on the soil type. Three soil types were treated with blood from an ASF-positive wild boar: beach sand, forest soil, and swamp mud. Beach sand showed high initial titers, but no infectious virus was recovered after three days. No infectious virus was recovered from the acidic forest soil even immediately after treatment, and the swamp mud had low titers initially but declined after three days. However, viral genome could be detected in all soil types throughout the entire observation period (Carlson et al., 2020).

Regarding measures taken to limit the spread of ASF throughout the European Union, the EU Commission publishes minimum guidelines for biosecurity plans. These guidelines include requiring ASF free certification for movement of semen and ova or the acquisition of new animals, visitor restrictions, and training for workers. Additionally, when hunting in regions where the virus is endemic or has a current outbreak, hunters must be trained in basic biosecurity protocols, evisceration is to be carried out with gloves and hands should be washed afterwards, any clothing worn during the hunt should be washed at high temperatures, and the hunters should avoid contact with domestic swine for 48 hours (Jurado et al., 2018a).

Swine experimentally infected with ASFV strains of varying virulence showed symptoms and seroconversion (with low virulence strain) prior to euthanasia. Animals were euthanized between 3- and 26-days post infection and samples were collected from multiple tissues and muscles. Frozen muscle samples were thawed prior to use and gently squeezed to extract exudate. Polymerase chain reaction (PCR) was performed on whole blood, muscle, and tissue samples, while ELISAs were run on serum samples. Cycle threshold (Ct) values from PCR closely correlated to ELISA results, with highly and moderately virulent strains resulting in lower Cts. Meat sample Ct values trended lower than the corresponding blood samples, and no genomic material was found in meat samples from pigs infected with the low virulence strain. The presence of ASFv genomic material in meat exudate likely corresponds to virulence, and in the absence of gold standard serum or whole blood samples, meat products such as those carried by airline passengers may be used to test for ASFV (Onyilagha et al., 2021).

The introduction of zoonoses or foreign animal diseases (FADs) into the United States via animal products carried by travelers is a real risk. On average, 8000 units of pork products are confiscated by Customs and Border Patrol (CBP) at U.S. ports of entry yearly. The sanitary status of these products is unknown, and they can easily carry diseases unbeknownst to the passenger. However, the rate of detection is typically under 50%, allowing the entry of a large quantity of pork products daily. Jurado et. al. built two statistical models to estimate the monthly probability of ASFV entry into the country. These models accounted for the number of pork products confiscated by CBP at airports and the number of passengers arriving in the U.S. via airplane at 87 international airports. No data was available on the exact country of origin of the pork products. For probability of ASFV entry, the high-risk airports were Dulles, JFK, Houston-Bush, Warwick, and San Juan, with Ghana, Cape Verde, Ethiopia, and Russia as the high-risk countries. May and July, two high-traffic summer months, carried the highest risk. Baggage inspection is limited by manpower and inspection times, and not every piece of luggage is inspected. Naturally, the risk of introduction increases during summer months with travel volume (Jurado et al., 2018b).

Currently, validated testing methods for ASF detection require animal restraint, such as nasal swabs and blood draws, or involve post-mortem tissue collection, as with spleen and tonsil samples. Oral fluid testing is often used for other swine diseases, including influenza A virus (IAV), PRRSV and porcine epidemic diarrhea virus (PEDV). Cotton ropes are hung in pens at shoulder height and animals are allowed to chew on them for 20-60 minutes, depending on prior experience. After the collection period, fluid is wrung out and stored for testing (Bjustrom-Kraft et al., 2018). Experiments seeking to validate the use of oral fluids for ASF detection were carried out at Plum Island Animal Disease Center (PIADC, USA) and the National Centre for Foreign Animal Disease (NCFAD, CAN), utilizing one animal per pen as a “seeder pig.” This pig was inoculated with ASF Georgia 2007/1 (highly virulent) or ASF Malta’ 78 (moderately virulent) and housed in a pen with 19-24 naïve pigs. Beginning on day 0 post-infection and continuing throughout the duration of the study, oral fluid ropes were placed in the pen and chewed for up to half an hour, then wrung out. ASF genomic material was found in individual samples as early as 3 days post-infection, and on every subsequent day until termination of the study as animals succumbed to disease. Further research into the viability of oral fluids as a testing method for ASF is crucial, as current methods of individual pig sampling are labor- and resource-intensive, while oral fluid is an aggregate sample that requires minimal manpower and fewer laboratory resources (Goonewardene et al., 2021).

1.1.3 Management: Disinfection and Survivability

Present ASFV management strategies are limited, as the mortality rate is high and there is no treatment. Disinfection of hard surfaces, such as pens and trailers, and decontamination of possible infectious materials, including soil and pork products, are the best methods of prevention. When beach sand and potting soil samples inoculated with infectious blood were treated with calcium hydroxide or citric acid, no virus was recovered after one hour of decontamination (Carlson et al., 2020). Application of lime as a disinfectant has also been effective in vitro, with three lime products showing a reduction in viral titer when applied to six German soil types. A 10% solution of either lime milk, slaked lime, or quicklime was sufficient to inactivate ASFV regardless of the water content of the soil. Additionally, previous studies demonstrated the efficacy of 0.1% peracetic acid in completely inactivating ASFV in all tested soil types, although it was limited by the presence of blood (Tanneberger et al., 2021). Lime was unaffected by the presence of blood, indicating its usefulness in areas surrounding infectious carcasses that are likely to be tainted by blood and other bodily fluids. Appropriate decontamination of the soil where infected animals died is critical in curbing further spread of ASFV, especially in countries such as Germany where the virus has spilled over into the wild boar population (Tanneberger et al., 2022).

Multiple methods of inactivation have been tested, including the use of a formaldehyde-based product (FBP) on ASFV in cell culture. ASFV titers were reduced by four logs in vitro after application of four concentrations of the FBP, although this does not necessarily reflect its efficacy in vivo, such as in commercial feed manufacturing settings (Trinh et al., 2022). When applied to 10^7 and 10^5 $TCID_{50}$ ASFV, highly complexed 5% iodine demonstrated significant inactivation of ASFV after five minutes of immersion or spray treatment, compared to commercially available 5% povidone-iodine, which did not inactivate ASFV until 15 minutes of immersion. Highly complexed 5% iodine therefore has the potential for use in industrial applications as a rapid disinfectant (Pan et al., 2021). Some commercially available disinfectants, including the liquid Virkon™ S have been tested against ASFV on surfaces commonly found in production facilities, such as stainless steel and concrete. Application of 1% Virkon™ S to inoculated concrete and stainless-steel sections resulted in complete inactivation of ASFV after a 10-minute contact time. Additional compounds tested in cell culture include sodium hypochlorite, glutaraldehyde, caustic soda, potassium peroxymonosulfate, phenol, acetic acid, and benzalkonium chloride. All seven of these compounds effectively inactivated ASFV at multiple concentrations recommended by the World Organisation for Animal Health (WOAH), although some were affected by the presence of organic material in highly soiled conditions (Juszkiewicz et al., 2020), demonstrating the importance of cleaning surfaces prior to disinfection.

Ozone is utilized in the food and medical industries as a disinfectant. It sterilizes surfaces quickly and produces oxygen as a decomposition product, and has inactivated RNA viruses in previous studies, with little work done to research its effects on DNA viruses. Porcine alveolar macrophages were inoculated with wild type ASFV and titered to ensure infectivity. ASFV isolates were incubated with ozonized water produced via an electrolytic ozone generator for 1, 3, 6, or 10 minutes prior to inoculation back into cell culture. Following further titers, it was determined that ozonized water reduced the concentration of ASFV by at least two logs at all time points (Zhang et al., 2020).

Research has shown that commercial curing and drying processes can inactivate multiple viruses, including ASFV. Although swine presenting with severe clinical signs at a slaughter plant would be rejected by the USDA after antemortem inspection, it was necessary to use these animals for processed meat products in this study to ensure virus presence in the selected tissues. Infectivity titers from inoculated pigs ranged from 5.4 to 9.5 -$\log_{10}$/mL, depending on the tissue. Shoulders, hams, and loins were cured according to traditional Iberian or Serrano methods and samples were collected throughout the process. ASFV was inactivated after 140 days in all cuts, indicating that the curing process is able to destroy the virus (Mebus et al., 1997).

Survivability studies have shown that ASFV is capable of surviving in various substrates for extended times at 4°C, including an estimated 112.6 days in hay and 97.4 days in straw. Leaf litter offered the lowest survival time at 18.9 days, while water and soil may sustain the virus for 35.6 and 32.8 days respectively. This study illustrates that contaminated surfaces, such as those where wild boar carcasses are recovered, may contribute to virus survival depending on the substrate. Additionally, ambient temperatures around 4°C will allow ASFV to survive for longer periods of time in certain substrates (Mazur-Panasiuk and Woźniakowski, 2020).

1.1.4 Management: Vaccine Candidates

African swine fever control, at present, relies heavily on destruction of all pigs inside of control zones and monitoring surrounding areas. Since there are no commercially available vaccines or treatments, mortality rates approach 100% in most areas. Multiple vaccine candidates are in progress, representing years of research across several countries. One such candidate is ASFV-G-ΔI177L, representing the deletion of gene I177L from the highly virulent ASFV strain Georgia. This previously undiscovered gene is conserved across multiple ASFV strains isolated from different countries, and animals inoculated with ASFV-G-ΔI177L did not develop clinical signs, remaining healthy during the 28-day observation period compared to animals inoculated with the parental strain (Borca et al., 2020). The vaccine developed from the altered strain shows protective capacity when administered via the oronasal route, compared to parenteral administration of other live attenuated vaccine candidates. Oronasal delivery is more feasible for use in wild animals versus intramuscular injection, and challenge studies showed no difference between delivery routes in protection from the parental ASFV-G strain (Borca et al., 2021). Additionally, this vaccine candidate effectively protects both European breeds and native Vietnamese pigs against the circulating Vietnamese field strain, with full protection accomplished by the fourth week post-vaccination.

Another gene-deleted strain, SY18ΔI226R, was developed in China. This gene deletion has never occurred naturally in circulating field strains, and the gene has been conserved with minor changes in all isolates, indicating it may play a role in maintenance of virulence. Removal of this gene did not affect replication in cell culture. After inoculation with SY18ΔI226R, study animals remained clinically normal. Following challenge with the parental SY18 strain, all animals in the control group developed clinical signs and three were euthanized prior to the end of the challenge period. Pigs in the inoculated group survived the challenge and only two developed low fevers. SY18ΔI226R-immunized pigs shed low numbers of virus on oral swabs, although the shed virus was found to be virulent. This is expected with live virus vaccines, although it presents a risk for environmental contamination and virus spread (Zhang et al., 2021).

1.2 HISTORY OF FOREIGN ANIMAL DISEASE OUTBREAKS IN NORTH AMERICA

1.2.1 Foot and Mouth Disease

The last occurrence of foot-and-mouth disease (FMD) in North America happened in Saskatchewan, Canada, in 1951. This outbreak was not recognized for three months as it was initially believed to be vesicular stomatitis (VS) after a horse inoculated with viral material developed vesicles on the tongue. The route of introduction is unknown but may be related to an immigrant worker from West Germany transporting the virus on his clothes or via a sausage product. Encompassing forty-two premises and resulting in the destruction of 4,077 livestock and 15,828 egg products, the epidemic had an economic impact of $722 million CAD after factoring in the year-long ban on exports. Documented incubation periods varied widely, ranging from three to 16 days. Unfortunately, many of the affected premises, including dairies, breeding farms, feedlots, and packing plants, likely shared workers and transported animals back and forth, making epidemiologic tracing difficult (Sellers and Daggupaty, 1990).

Further complicating the 1951 outbreak was possible airborne spread from pigs also housed on feedlot 4 (Daggupaty and Sellers, 1990). Given the original diagnosis of vesicular stomatitis, animal health officials likely did not consider the pigs to be important in the disease investigation, considering the rarity of VS in swine (Hanson, 1952), and FMD in pigs was previously difficult to detect for less experienced samplers (Sellers and Forman, 1973). Calculations using a short-range Gaussian plume dispersion model demonstrated that feedlot 4 was the only likely source of the large quantities of airborne virus required to infect farms 6, 7, 8, 10, and 16.

The U.S. has been FMD-free since the control of a 1929 outbreak in Kansas, but the threat of its reintroduction looms. In a modeling exercise involving a widespread FMD outbreak, the resulting losses to the U.S. economy were estimated at $11.7 billion USD, with losses to the rest of the world from trade restrictions estimated at $14.1 billion USD (Boisvert et al., 2012). Traditional "control" of an outbreak requires total depopulation of any infected premises and close monitoring of neighboring locations, typically followed by heavy economic impacts due to the cost of destruction of animals, production losses, and loss of export markets. Alternative eradication methods have been studied, including slaughtering only contagious herds vs. those in contact with contagious herds, and vaccinating all animals in a certain radius of contagious herds. Overall, preemptively slaughtering all herds in contact with known infectious premises was less costly than using a ring system to determine which herds were more likely to be infected. Early ring vaccination decreased the duration of outbreaks in the simulation, but this method was more costly if vaccinated animals were ultimately slaughtered due to their herd contact status (Schoenbaum and Disney, 2003).

Global eradication of FMD is a distant goal of the Food and Agriculture Organization (FAO), but unlike some diseases that have a reliable vaccine and do not cause persistent infection, FMD has seven antigenically different serotypes that are clinically identical and can result in lifelong infection. During active infection, the virus is secreted in all bodily fluids, including breath. FMD-free countries typically rely on strict import and animal contact controls, although this method has failed in the past, resulting in an outbreak that cost the United Kingdom $12 billion USD and forced depopulation on a scale now known to be publicly unacceptable. Early vaccination is encouraged to reduce outbreaks, even though vaccine immunity lasts approximately 4-6 months and protection wanes as the outbreak strain gains antigenic distance from the vaccine strain. Eradication is a lofty goal, and current FMD vaccine technology is not sufficient to act as a barrier to outbreaks (Kitching, 2005).

1.2.2 Highly Pathogenic Avian Influenza

The first mention of highly pathogenic avian influenza (HPAI) dates to Italy in 1878. Since then, the H9N2 and H5N1 strains have both been transmitted to humans and become endemic in Asia. In the U.S., the first known outbreak occurred from 1924-1925, beginning in live animal markets in New York City, then spreading along the eastern seaboard. Cases were found in the Midwest near the end of the outbreak, likely stemming from contaminated rail cars traveling west from the east coast. This outbreak and later infections of several flocks in New Jersey in 1929 were contained through a combination of depopulation, quarantine, sanitation, and halting live bird movements, coming at a cost of $1 million USD. An early 1983 outbreak in Pennsylvania began with a low pathogenic strain that mutated to become highly pathogenic, forcing the culling of 17 million birds and carrying a total cost of over $300 million USD. Canada fell victim to another instance of mutation from low to highly pathogenic in 2004, with over 400 commercial poultry farms and 550 backyard flocks depopulated to curb the spread. Additionally, two depopulation workers were confirmed infected with HPAI during eradication (Lupiani and Reddy, 2009).

Surveillance for HPAI is ongoing throughout the U.S. These efforts began in 2006 as an interagency effort to determine whether wild bird populations harbored HPAI. At the time, the greatest concern for introduction of HPAI was via illegal imports of live birds and poultry products, with wild bird migration as a secondary concern. As wild birds travel through flyways, they cover large geographic areas and can interact with captive birds, spreading a multitude of diseases. HPAI has up to a 100% mortality rate in domestic birds, but it is often asymptomatic in non-domestics. Oropharyngeal and cloacal swabs were collected from hunter-harvested and live-caught birds, environmental fecal samples, and any birds involved in a morbidity or mortality event. National Animal Health Laboratory Network (NAHLN) labs screened all samples, and positives were shipped to the National Veterinary Services Laboratory for confirmation via virus isolation. Hot spots were located primarily in the North, particularly in regions known as migratory staging areas, such as the Delaware Bay and the Prairie Pothole region. Prevalence of AIV was the highest in dabbling ducks, which accounted for 86.4% of positive samples, and the mean prevalence increased each year despite sample numbers decreasing due to limited funding (Bevins et al., 2014). No HPAI was found in the wild bird population, but the massive sample numbers generated from these surveillance efforts provided important insight into the evolution and ecology of influenza in these birds.

Prior to the ongoing HPAI outbreaks, which are currently affecting 47 states and over 58 million birds between 321 commercial and 468 backyard flocks (USDA APHIS | 2022-2023 Confirmations of Highly Pathogenic Avian Influenza in Commercial and Backyard Flocks), the largest outbreaks in the U.S. happened in 2015, with over $3 billion in economic impacts. Producers controlled the disease after significant culling, but the route of transmission was not easily determined. Some farms reported abnormal bird mortality immediately next to air intake vents and traditional biosecurity methods were unsuccessful in preventing sickness, suggesting airborne transmission (Zhao et al., 2019). Previous studies demonstrated respiratory droplet transmission of avian influenza H5N1 to ferrets (Herfst et al., 2012; Imai et al., 2012). Zhao et. al. found that many of the affected Iowa farms could have received infectious virus from contagious farms in Minnesota and South Dakota within an 8-hour air radius. Ninety-seven percent of the Iowa cases were proximate enough to have received or contributed to intra-state airborne transmission, and seven different states may have added to the inter-state transmission. Most poultry farms could reduce the severity of future outbreaks with the addition of air intake filtration systems, which some producers are proactively adding to their housing facilities (Zhao et al., 2019).

1.2.3 Classical Swine Fever

Classical Swine Fever (CSF), also known as hog cholera, is caused by the classical swine fever virus (CSFV), an enveloped RNA virus in the *Flaviviridae* family and is a WOAH-reportable transboundary animal disease. CSF has not been reported in the U.S. since 1978. The eradication of CSF in the U.S. was successful due to more than 60 years of research on the virus and management tools, regulatory decisions to control disease spread, and partnerships between private and public sectors of the swine industry (Shi et al., 2021). The virus is closely related to bovine viral diarrhea virus and ovine border disease virus. Domestic and feral swine, as well as wild suids like javelina, are susceptible to this virus and it can be transmitted through direct or indirect contact with mucosal surfaces, in infected feed components or pork products, and vertically from a sow to her piglets. A CSF infection can be chronic, acute, or prenatal. Piglets infected in utero develop a fever, anorexia, respiratory symptoms, and ataxia before succumbing to the illness within three weeks after onset (Brown and Bevins, 2018b). Older pigs may survive for 2-3 months after infection but will persistently shed virus during that time. Highly virulent strains are shed in large quantities through all bodily fluids, while less virulent strains are found primarily in oronasal secretions (Weesendorp et al., 2009).

Given the potential impacts of a new outbreak of CSF in the U.S., it is important to understand the relative risk of importing live animals or pork products. Using stochastic risk assessment models, Herrera-Ibatá et al. estimated the probability of introducing ASFV or CSFV through imports. The models considered three steps: entry, exposure, and consequence assessments. Iowa, Minnesota, and Wisconsin were the highest-risk states, representing 57% of the probability of CSFV introduction. A vast majority of imported live pigs, 99%, come from Canada, and most other countries have a near-zero risk factor for imports. Regarding the legal import of pork products, Florida, Illinois, and California posed the greatest risk, with products likely coming from Finland, Canada, and the Cayman Islands. According to these models, the risk of CSFV introduction in swine products was twice as high as the risk of ASFV introduction, but swine products in general presented a risk six times lower than that of live pigs. Such risk analyses aim to provide importing countries with the data necessary to restrict or halt imports of animals or animal products from certain countries or regions. The risk of feral swine exposure to CSFV-contaminated garbage products was not evaluated, but the potential for swill or garbage feeding to spread the virus was 64% more likely if the waste came from households, rather than commercial retailers or restaurants. In order to minimize this risk, any swill fed on swine operations should be appropriately heat treated prior to use (Herrera-Ibatá et al., 2017).

Beyond the known risk from the *legal* import of swine products, it is also important to discuss *illegal* products smuggled into the country. This includes undeclared products in air passenger luggage. Out of all agricultural items confiscated by USDA APHIS at any U.S. ports of entry between 2005 and 2006, 30% were animal products, including 8,000 units of swine products. Breaking this down further to include only the items confiscated at international airports, 45% of illegal pork products were discovered in personal luggage. The greatest number of confiscations occurred in July. The mean annual probability of CSFV entry on smuggled pork products was 0.414, which roughly equals one entry every 2.5 years, greater than seven times the risk of ASFV entry. Six states and territories represented 79% of the total annual risk, and the Dominican Republic and Cuba posed 90% of the risk as countries of origin. Understanding the risk of FAD incursion through ports of entry will help shape the U.S. response in the face of a potential outbreak in a neighboring country (Jurado et al., 2018b).

Although it is impossible to stop all potential routes of FAD entry into the U.S., careful assessment of the highest risk ports of entry can help to prevent outbreaks while our pork production system ramps up preparedness and bolsters itself against disruptions. There is not one most likely route of entry for either CSFV or ASFV, but these modeling studies offer a better understanding of the risks and benefits of importing live animals and pork products as well as halting smuggling of illegal swine materials.

1.3 SWINE FARM BIOSECURITY IN THE UNITED STATES

1.3.1 Current Practices: Commercial Operations

Biosecurity on commercial swine production facilities specifically refers to the prevention of introduction of infectious agents. The term was relatively new to the industry in the early 2000s and has quickly become an everyday consideration for commercial producers of all levels, from boar studs to finishing barns. Any immunologically naïve herd faces greater potential risk in the event of an outbreak, as these animals have never been exposed to most diseases. The biggest concerns for pathogen spread are the introduction of infected animals to new facilities and the concentration of swine facilities in any given area, and as such regions with a higher density of swine farms must follow stricter biosecurity guidelines (Amass and Clark, 1999).

Prepared feed and feed ingredients intended for swine consumption have been shown to harbor pathogens and may transfer diseases to otherwise bio-secure premises. When viruses such as ASFV, CSFV, IAV, and their surrogates were used in models mimicking trans-Atlantic and trans-Pacific shipping, some could be recovered from up to 10 out of the 11 substrates tested. ASFV remained stable in 9 substrates with a half-life of 1.3-2.2 days, shorter than other viruses in the study but consistent across ingredients. The ingredients that most frequently supported virus survival included soybean meal, lysine hydrochloride, and complete feed. Conventional soybean meal also had the highest percentage of moisture at 12%. Overall, survival was variable and depended heavily on characteristics of the feed ingredient and the virus itself. ASFV was the only virus to survive in the control matrix. This may suggest that the feed ingredients protected other viruses from the temperature and humidity fluctuations in the simulated shipping scenario, compared to the propylene tube with stock virus as a control (Dee et al., 2018).

Further research into the half-life of ASFV in shipped feed with more time points demonstrated that the half-lives in most ingredients were longer than seen previously. With five nodes representing critical points in the feed production and shipping process (ASFV contamination of raw materials, inactivation during processing, recontamination after processing, inactivation during transit, and inactivation during entry into the U.S.), this updated model found that the median annual risk of contaminated shipments of corn entering the U.S. was 2.0%. Other feed ingredients had a lower risk of both initial contamination and recontamination after entry. These other ingredients, such as soybean meal, are typically extruded or solvent extracted prior to import, and these processes reduce the likelihood of virus survival (Schambow et al., 2022).

Extended transport time also correlated with decreased risk, as the longer time in shipment increases the chance of virus inactivation. However, there is a significant knowledge gap in the specifics of ASFV prevalence in feed ingredients due to a lack of surveillance at import. ASFV transmission through infected feed or bedding has not been thoroughly studied, and may become critical in the fight against the virus moving forward as we look to maintain and improve biosecurity at the farm level (Shurson et al., 2022).

Emphasizing the importance of disease surveillance in feed ingredients, a 2014 PEDV outbreak on an Ohio swine farm was eventually traced back to contaminated feed despite their appropriate biosecurity protocols. The facilities had no visitors within 10 days of the outbreak, and after a thorough review of employee movements, there were no trips to foreign countries. All employees and non-employee contractors were required to follow the company's biosecurity protocols to enter and exit the facility. Only managers were permitted to move between sites, and these sites were all within the same production flow. These facilities had been free of PRRS or PEDV for over seven years. Supplies such as veterinary materials and semen were assessed for potential contamination, but these items were ruled out as they were not shared between production flows and were disinfected in a fume chamber prior to intake. Additionally, aerosols and droplets likely did not contribute to disease spread in this scenario, because affected sites were not located close enough to each other for aerosol transmission to occur. Immediately prior to this outbreak, the company switched to a new supplier for starter pellet feed, and PEDV genetic material was found in this feed after this extensive epidemiologic investigation. These starter pellets were disinfected in the same manner as veterinary supplies, eliminating contamination on the surface of the bag but not affecting the pellets inside. Production sites within the same flow that never received the new feed remained PCR negative (Bowman et al., 2015). Further investigation is needed to determine the true infectivity of feed and appropriate methods of decontamination.

In commercial swine production, animals may be moved to different facilities depending on their age and production status. This may involve moving weaned pigs to a grower barn or shipping cull sows out of a breed-to-wean facility, typically within the same "flow" or group of barns encompassing all stages of production. Many companies have dedicated trailers for each facility and will isolate cull trailers completely since these trucks travel to collecting points and livestock auctions where diseases are prevalent. Scale model livestock trailers were experimentally contaminated with PRRSV-spiked feces to simulate the conditions of vehicles after moving pigs, then underwent a high-pressure wash like that performed in industrial truck washes. The final step was disinfection with either accelerated hydrogen peroxide (AHP) or glutaraldehyde combined with quaternary ammonium (GQA) depending on the treatment group. The pressure washing alone reduced quantities of PRRSV genomic material despite the presence of visible fecal material remaining after cleaning, but samples collected from control trailers still resulted in infections when inoculated into live animals. Both disinfectants destroyed viable PRRSV and samples taken from these trailers after a 15-minute contact time did not infect study animals (Schneider et al., 2015).

In a study examining the efficacy of a peroxygen-based disinfectant, diamond aluminum coupons intended to replicate trailer surfaces were contaminated with PEDV-spiked feces, then disinfected with one of 10 treatments. These treatments used the same disinfectant at different concentrations, temperatures, and contact times, with phosphate buffered saline (PBS) as a control. Following treatment, environmental samples were taken from each coupon and inoculated into a randomly-selected, PEDV-negative barrow. Results showed that the disinfectant was highly effective in winter-like conditions (4°C and -10°C) after a 30-minute contact time, when applied to low levels of organic matter. This is important in the event of less-than-ideal washing, which may occur particularly in the winter, as the disinfectant was still effective against PEDV (Baker et al., 2018). The combination of effective cleaning and a disinfectant given the correct contact time can reduce incidence of disease, particularly of viruses that may be carried on trailers returning to their “home” sites.

Breed-to-wean production sites, where young pigs are born and raised until weaning when they are transferred to a grower facility, fit a critical niche in the U.S. pork production system. However, they also have a unique role in disease transmission, since young pigs can contract, incubate, and spread diseases like influenza A (IAV) to other farms after weaning. In an effort to understand the IAV burden in suckling pigs, 83 breed-to-wean farms voluntarily shared their farm surveillance data and breeding animal vaccination status. During the six-year period, 23% of the 12,814 samples were RT-PCR positive. Following a season-adjusted multivariate analysis, vaccinating sows against IAV and ensuring gilts were IAV-negative at intake were the only measures to significantly reduce piglet infections at weaning (Chamba Pardo et al., 2018).

Regarding specific knowledge on the exact day-to-day biosecurity and management protocols followed by swine producers and the extent to which these producers and their employees understand such recommendations, little scientific literature exists. However, the National Pork Board and Pork Checkoff have published ample materials targeting lay swine producers with audience-specific posters, articles, and other resources in this space. These resources include posters with photographs of good and bad biosecurity practices, articles about steps to take after international travel, and booklets containing basic information on common swine diseases (NPB Brand Portal). Both websites also promote the Secure Pork Supply (SPS) program, a collaboration between industry partners, state and federal animal health officials, Iowa State University, and the University of Minnesota. SPS plans aim to allow continuity of business in the event of FAD incursion, helping producers to prove that their animals are disease-free for movement purposes and to continue limiting their exposure (SPS Plan).

1.3.2 Current Practices: Niche and Hobby Farms

The exhibition swine population is concentrated in Ohio and Indiana, unlike the commercial population. Surveys conducted in 2017 reported an average of 226.8 pigs per county fair in Indiana (Bliss et al., 2017), a majority of which are sourced from niche and hobby swine breeders. Minimal scientific literature exists on the exact biosecurity and animal management practices followed by niche and hobby producers on their own premises, including exhibition swine breeders. However, these animals are frequently transported to exhibitions and sales in different areas of the country, and research has been conducted on the contribution of movement patterns to disease spread at these events.

A study carried out at the 2005 California State Fair surveyed 137 exhibitors showing all livestock species and representing 40 of the state's 58 counties. Out of the 72 hogs represented in the survey, 90% of market and 100% of breeding animals were planned to return home following the show. Biosecurity precautions were minimal, with 7% of respondents indicating that they did not follow any precautions prior to the exhibition and 10% reporting no major precautions during the exhibition. For all species, the most common guidelines followed were no equipment sharing and no direct physical contact with other animals. In general, low quarantine rates were reported and 57% of respondents intended to return their animals to a commercial livestock facility. This combination of minimal quarantine (shorter than the incubation period for most illnesses) and return to commercial facilities demonstrated a significant risk for disease outbreaks should these animals be exposed to pathogens at the fair (Thunes and Carpenter, 2007).

Minnesota ranks in the top 5 pig-producing states in the nation, yet its exhibition swine population remains largely uncharacterized, like that of other major pig-producing states. A 2012 study collected blood samples from 661 exhibition pigs at slaughter and gathered surveys and interviews from 4-H participants who registered a swine project for their county fairs. Only 9% of fair boards included in the survey required that all animals were sent to slaughter at the conclusion of the show, a measure that can reduce disease spread by preventing the return of animals to production herds. This is particularly important because the population of 4-H swine significantly correlated to the commercial swine population at the county level, with 36% of respondents raising commercial pigs on the same premises and 15 individuals keeping commercial and exhibition swine in the same barn (Wayne et al., 2012). In contrast, only 13.3% of exhibitors in Ohio and Indiana reported raising commercial and exhibition swine on the same premises (Bliss et al., 2017).

The implications of a showpig being exposed to diseases at a county fair and returning to the same premises or barn with a commercial swine herd are immense. Respondents were presented with a list of biosecurity recommendations and asked to mark them as “important,” “unimportant,” or “unfamiliar.” The top three recommendations rated as important were transport sanitation, sourcing healthy pigs, and separating unhealthy pigs. Participants rated “shower upon entry and exit,” “maintain visitor log,” and “wear mask and gloves” as the least important and were the least familiar with recommendations to provide boot baths and bird-proof barns (Wayne et al., 2012).

Blood sample results showed that seroprevalence for PRRSV in the Minnesota showpig sample was approximately 48%, making exhibition swine a potential reservoir for the disease. However, given the much larger population of commercial animals in Minnesota, the risk for disease spread is likely greater from commercial swine to show animals, rather than the other way around. Unfortunately, it is impossible to evaluate the exact risk posed by exhibition swine due to the transient nature of their population, being purchased in the spring and usually sold or butchered after the county fair in the summer. Youth exhibitors who participated in this study were members of 4-H and many also held membership in FFA chapters, opening possible routes for education on infectious disease and biosecurity as clubs and chapters prepare for the show season (Wayne et al., 2012).

Swine influenza has been associated with over 400 zoonotic IAV infections from county fairs since 2011, emphasizing the importance of fully understanding exhibition animals' role in disease spread. While some county fairs are fully terminal and require shipping all market animals to slaughter, "jackpot" circuits held earlier in the year are never terminal and facilitate mixing of animals from different farms for several days at a time before returning home (Bliss et al., 2017). These circuits may serve as a source of the reassorted IAV strains that later infect humans at county fairs. When jackpot animals were tested starting in April, IAV first appeared as a PCR positive in May in the state circuit, then in a June national jackpot, then throughout the duration of county fair season from June to October. IAV was detected at significantly higher levels at jackpot shows (76.3%) compared to county fairs (37.8%). Additionally, the virus was detected 68.9% of state shows and all national shows sampled, regardless of the density of the local swine population. Given the potential for exhibition animals to be infected with or exposed to diseases at shows or fairs, implementation of appropriate biosecurity measures at these events is important in preventing disease transmission between animals and zoonotic transmission to humans (McBride et al., 2021).

Representing a significant contrast from commercial operations, niche and hobby swine breeders often host "open barns" at their farms prior to sales, which may also be hosted onsite. Open barn events invite members of the public who are interested in buying pigs, typically for their youth exhibitors or their own breeding stock, to visit the farm and evaluate pigs in person.

Farm owners may require the use of boot covers and coveralls, but visitors do not normally shower in and out or limit contact with other swine before or after the event. These pigs are also transported and commingled with other pigs many more times in their life compared to commercial swine. Some exhibitors reported attending as many as 50 shows before their county fair (Bliss et al., 2017).

Although fairs and exhibitions may use signage and announcements to recommend biosecurity measures to exhibitors and members of the public, such recommendations are not always heeded or even viewed as important by individuals in livestock areas. This disregard for biosecurity leads to outbreaks of zoonoses and animal infectious diseases, such as a 2016 H3N2v outbreak linked to agricultural fairs in Ohio and Michigan that sickened 18 people, including seven children under the age of five. Every case reported exposure to swine at one of the seven fairs, and no person-to-person transmission was found. All individuals fully recovered, and pigs at all seven fairs tested positive for H3N2 (Schicker et al., 2016). Concurrent with this outbreak, swine exhibitor households participating in eight Michigan fairs were asked to complete surveys exploring their knowledge of and support for various biosecurity recommendations. Despite evidence to the contrary, 90% of respondents perceived their risk of contracting influenza from swine to be low or very low. Exhibitors generally did not support closing barns to the public, holding "distance" swine auctions, or reducing swine shows to under 72 hours to minimize risk of disease transmission, but indicated overall support for better monitoring of hand wash stations outside of barns (Stewart et al., 2018).

Many county and state fairs require livestock animals to remain on the grounds for over a week depending on the length of the exhibition, regardless of when in that timeframe these animals pass through the show and sale rings. Keeping animals on the grounds and allowing them to interact with each other for extended periods of time exacerbates disease spread, compounded by suppressed immune systems due to the stress of moving into the fairgrounds. This phenomenon led to the "72-hour rule," a suggestion for fair boards to release or sell all swine within 72 hours after they arrive on the grounds. One 2014 fair had 3.8% IAV prevalence at 15 hours after arrival and 77.5% prevalence after 150 hours. Fairs tested in 2018 and 2019 were asked if they released some, all, or none of their pigs before 72 hours, and the mean prevalence in fairs releasing all pigs (6.1%) was significantly lower than fairs releasing some (33.2%). It is expected that some animals will arrive carrying diseases, but implementing measures such as the 72-hour rule at a fair level removes the ability for individual exhibitors or families to ignore one particular biosecurity recommendation (McBride et al., 2022)

Swine exhibitions are an important piece of agricultural fairs, and the life skills that youth learn through participation in such events are invaluable. However, it is undeniable that the exhibition swine population serves as a reservoir for important diseases such as IAV, and their caretakers may not be fully aware of or willing to follow the biosecurity recommendations that have been implemented by commercial swine producers for years. Moving forward with U.S. foreign animal disease prevention and preparedness, it will be critical to have a comprehensive understanding of niche and hobby swine producers' and exhibitors' knowledge of and attitudes towards biosecurity principles.

1.4 YOUTH BIOSECURITY BASELINE

1.4.1 Perceptions and Understanding

Considering the ongoing HPAI outbreak in 47 states that has affected over 57 million domestic poultry to date (USDA APHIS | 2022 Confirmations of Highly Pathogenic Avian Influenza in Commercial and Backyard Flocks), it is more important than ever that all youth, especially those involved in agriculture, are well-educated in biosecurity principles. Before attempting to offer educational programs, we need to assess the baseline of perceptions and understanding for youth livestock exhibitors to avoid providing materials that are too far above or below the appropriate level. Most scientific literature in this space pertains to biosecurity practices followed at exhibitions rather than broader understanding of the principles, and rely heavily on self-reporting through surveys, but these studies do evaluate basic knowledge of the concept and recommendations.

Families exhibiting livestock in 4-H classes at the Kansas State Fair were asked to answer a series of questions about their youth exhibitors' habits before, during, and after the fair. The results covered all species, with some questions broken down by specie. Most exhibitors, 80.4%, reported checking animals for disease prior to moving in, while only 11.1% claimed to disinfect footwear before the fair. Among swine exhibitors, 12.9% never disinfected equipment before traveling to a fair and 30.6% never disinfected shared equipment before using it on their own animals. Nearly 70% of all exhibitors said they do not quarantine animals after returning from an exhibition. The results demonstrate a general lack of understanding of disease transmission and the importance of frequently disinfecting equipment, shared or not. This survey was, however, limited in its scope due to the exclusion of FFA members and out-of-state exhibitors (Larson, 2017).

The document "Measures to Minimize Influenza Transmission at Swine Exhibitions" (MtM) is published yearly by the CDC in collaboration with human and animal health officials, with recommendations including "clean and disinfect all tack and equipment between shows," "wash your hands with soap and water when you leave the barn," and "no food or drink in animal areas." Nolting et. al. aimed to evaluate the impacts of this document on youth swine exhibitors, particularly those exhibiting outside of county fairs alone. Each recommendation put forth in MtM was followed by statements asking about exhibitors' awareness of, opinion towards, and behavior inspired by the recommendation. Some were already widely followed, such as preventing sick pigs and people from attending shows and disinfecting equipment between shows, but those least likely to be followed were no eating or drinking in barns and isolating animals after returning from a show. Nearly 80% of exhibitors eat or drink in the barns and half reported sleeping in animal areas (Nolting et al., 2019). These results indicate some awareness of biosecurity principles and recommendations, but there is resistance to following guidelines even if they are shown to reduce disease transmission.

1.4.2 Education Efforts

Many education efforts surrounding biosecurity and disease prevention are geared exclusively for youth livestock exhibitors and are delivered through 4-H and FFA programs. However, there are some external efforts attempting to enhance these programs and evaluate their efficacy. Beginning with 4-H and FFA-based efforts, Minnesota 4-H Animal Science partnered with researchers from the University of Minnesota to develop modules for nine species to be offered at state shows and day camps. The group developed the Biosecure Entry Education Trailer (BEET) as a mobile classroom and taught lessons about disease prevention and biosecurity at each show or day camp. The BEET included a mock barn entryway and participants were taught the proper methods for crossing a line of separation to enter a biosecure facility. Each participant completed pre- and post-workshop surveys the same day and received an electronic survey six months later to evaluate the long-term impacts of the events. According to the post-workshop surveys, swine exhibitors strongly agreed that they could write a biosecurity plan after the event and saw a 36.5% gain in overall knowledge. Swine and poultry exhibitors were the most comfortable with biosecurity concepts, likely due to the prevalence of outbreaks in their respective industries (Schuft et al., 2022). Strengths of this format include the hands-on activities, small groups to allow higher-quality interactions with the facilitator, and take-home materials in a biosecurity kit.

One benefit of enhancing youth biosecurity education is the positioning of these youth as "experts" in their communities after the programming. This helps to shift power dynamics and empowers young people to lead (Calabrese Barton et al., 2013). California 4-H members recruited by county representatives participated in the "4-H Bio-Security Proficiencies Program," a multi-week workshop consisting of in-person and distance learning modules. Following completion of all modules and evaluations, the students acted as citizen scientists by developing biosecurity improvement plans for the Yuba-Sutter fair. The recommendations in these plans were considered by the fair board and the students reported which improvements were made, including new handwashing stations and removing the mixed species "champions row." During the year following their county fair, the 4-H members engaged with the UC Davis Veterinary Medicine Extension team to develop educational videos targeting fair leadership, youth exhibitors, and the public. After these community science activities, students were able to identify the changes they had made, they could watch themselves teach others about biosecurity, and they understood the significance of their roles as citizen scientists (Smith et al., 2021). The California program was successful for the same reasons as the BEET team, in that it offered students the chance to not only learn about biosecurity but to apply it in a very meaningful way for their community.

Many external education efforts, although not channeled directly through 4-H or FFA programs, still rely heavily on the participation of these members at their county fairs or jackpot shows. Five Ohio counties were chosen for their existing participation in IAV surveillance and greater numbers of enrolled swine exhibitors. During required quality assurance training sessions, research staff added tabletop scenarios designed to demonstrate appropriate animal management before, during, and after exhibitions to reduce IAV transmission. The scenarios were adapted from activities conducted by the Ohio Department of Health during zoonotic disease response trainings. Exhibitors completed a pre-test, reviewed a short lesson on IAV and disease spread, then broke into small groups to run through an assigned scenario before taking the post-test. The activities helped youth understand viral transmission between animals and between animals and people, as shown by the score increases of 1.54 and 1.78, respectively, on the relevant questions (Nolting et al., 2018). This teaching method was effective in its niche but would likely see broader success when combined with a truly hands-on activity like the BEET lesson or community science. Incorporating multiple teaching methods in a One Health approach was the basis for the Healthy Animals / Healthy YOUth program that distributed 120 resource kits and was offered to over 5,000 youth in Virginia and Maryland (Ashby et al., 2021).

1.5 ADULT BIOSECURITY BASELINE

1.5.1 Perceptions and Understanding

The broad focus of past research in the biosecurity and infectious disease space has been on youth exhibitors and agriculture students, with minimal studies on the baseline knowledge, perceptions, and understanding of adults involved in agriculture. Agricultural educators, 4-H leaders, and youth development and natural resources Extension agents are all likely to interact with youth on a near-daily basis for educational purposes. These groups tend to be aware of zoonotic diseases that they could contract from animals and know basic precautions like using PPE, but they were less aware of or knowledgeable about their own ability or responsibility to educate others on zoonotic diseases. Respondents were able to identify rabies, salmonella, and ringworm as zoonotic, but incorrectly chose hepatitis and distemper from a list of diseases. Despite a lack of confidence in their ability to teach others about biosecurity or zoonoses, these individuals believe 92% and 87% of the teaching responsibility for these topics should fall to agriculture teachers and Extension agents, respectively. Many respondents report never receiving formal training or education on zoonoses, but were able to find information easily and knew how to use these resources (Holub, 2017).

With this lack of training and minimal confidence in their own knowledge, it is natural to assume that educators rely on outside curriculum. However, some instructors voiced their concerns about a lack of age-appropriate materials for their agriculture classes, instead sourcing community knowledge by bringing in guest speakers and successful producers. In the absence of useful curriculum, these educators turn to their own experiences and as a result, food animal production lessons, including biosecurity, can be highly varied even within the same county (Jarvis, 2020).

There is a significant lack of knowledge about baseline biosecurity and infectious disease perceptions and understanding among adults in agriculture, despite these adults being expected to teach the next generation. It is important to focus not only on offering youth educational programs in this space but also on tailoring programming to meet the needs of adult agricultural educators to better serve their students and advisees.

1.5.2 Education Efforts

Minimal peer-reviewed literature examining education efforts for adults in agriculture exists. One such study incorporated One Health principles into Train-the-Trainer workshops for 4-H staff, Extension volunteers, agricultural educators, and livestock show officials in Virginia and Maryland, reaching 94 facilitators (Ashby et al., 2021). This is only a small component of a safe, healthy livestock industry, but little is known about outreach and education efforts for adults.

CHAPTER II: EVALUATING YOUTH AND ADULT PERCEPTIONS AND UNDERSTANDING OF AFRICAN SWINE FEVER AND BIOSECURITY IN THE EXHIBITION SWINE INDUSTRY

2.1 INTRODUCTION

Over 58 million poultry have been depopulated amidst the ongoing outbreak of highly pathogenic avian influenza (HPAI). All 50 states have wild bird cases, and 47 states are impacted by poultry cases (CDC, 2023). This epizootic, including the economic impacts seen after depopulation of so many birds, closely resembles a hypothetical future incursion of African swine fever (ASF) into the United States. If ASF breaches U.S. borders, feral swine would likely act as reservoirs and vectors for the virus and transmit it to commercial and exhibition swine as they move through their territories. Exhibition swine are animals produced specifically for evaluation by judges at dedicated shows, such as agricultural fairs that facilitate commingling of animals from many premises for extended periods of time (Bowman et al., 2012). These animals, following their current expansive movement patterns for shows (Bliss et al., 2017), could spread ASF across the country in a matter of weeks as they attend county, state, and national shows.

Two large associations, the National Junior Swine Association (NJSA) and Team Purebred, have a membership body of over 15,000 youth under the age of 21 who show pigs through these organizations. Most youth exhibitors have a strong support network of adults, often including their parents or guardians, the producer of their animals, local experts, and "fitters" or those who prepare animals for the show ring professionally. However, little is known about the basic knowledge and understanding of ASF and biosecurity guidelines in either of these groups, across the spectrum from youth to adults. As the U.S. pork industry enhances foreign animal disease prevention and preparedness efforts through the U.S. Swine Health

Improvement Plan (U.S. Swine Health Improvement Plan) and Secure Pork Supply plans (SPS Plan), it is critical to determine the knowledge gaps in the exhibition swine sector, as this segment of the industry is one of the biggest threats to commercial animals. After the baseline knowledge & understanding is gathered, educational activities can be developed and tailored to fill the current gaps in infectious disease outreach and teaching.

2.2 MATERIALS AND METHODS

2.2.1 Study Population

This survey intended to collect the baseline knowledge, perceptions, and understanding of ASF and biosecurity principles from youth exhibitors at all levels of exhibitions. Any youth residing in all states showing pigs at all levels of competition, including county and state fairs, local jackpot circuits, regional shows, and national jackpots were welcome to participate. There is no minimum age to show swine through NJSA or Team Purebred, but members are required to be capable of showing their animals without any assistance. Children showing at county fairs are required to be 8 years of age or older (4-H Programs). However, parents were allowed to help their children read this survey, thus the lower age cutoff was 3 years.

The adult survey's goal was to capture a baseline for ASF knowledge, perceptions, and understanding, as well as general knowledge of biosecurity principles and recommendations to enhance knowledge on these baselines to fill future educational and outreach needs. Any individuals who were involved with the showpig industry in some way, but not showing pigs themselves at the time of data collection were broadly targeted for this study population. This included, but was not limited to, parents of youth exhibitors, niche and hobby breeders, judges, fitters, extension agents, club advisors, and agricultural educators. Individuals living in all states and participating in any level of competition were invited to participate. Advisors and educators were asked to respond even if they had as few as one student showing pigs.

2.2.2 Survey Development

Two surveys were created on the Qualtrics experience management platform (Qualtrics LLC; Provo, UT) for distribution to youth and adult participants. Approval was obtained from the Colorado State University Institutional Review Board (IRB) for protocol numbers 3067 (youth, expedited) and 3075 (adult, exempt). The youth survey (appendix A) screened participants by age and parental consent, asking first if respondents were over the age of 18 and if no was selected, they were asked to obtain consent from parents or guardians before proceeding.

The youth survey began with a series of seven questions designed to capture perceptions of ASF and biosecurity. Question types included multiple choice, Likert scale, and dichotomous. Topics ranged from general understanding of biosecurity principles to methods of disease spread and the types of swine ASF infects. Some questions were adapted from a 2018 study in which youth exhibitors were asked about their knowledge of influenza A and basic biosecurity before and after a brief educational presentation (Nolting et al., 2018). It is important to note that the Likert questions asking for ratings of biosecurity understanding and concern regarding ASF were self-estimations, and previous research has noted that allowing study participants to rate their own abilities and knowledge may result in overestimation compared to objective measurements (Kruger and Dunning, 1999; Ackerman et al., 2002).

Respondents were then directed to four demographic questions where they chose their age from 3-21 years, which (if any) swine organizations they held memberships with, and their state of residence. The survey then split respondents into separate question flows based on their answer to the question “Have you traveled out of your home state for a swine show within the last year?” If yes, the following six questions captured information about which states they visited, what type of shows (terminal or non-terminal, county/state/national) they attended, and the biosecurity protocols they followed before, during, and after the shows. If no, the subsequent question flow asked similar questions about shows attended within the state of residence.

Similarly, the adult survey (appendix B) included the same knowledge and perception questions as the youth survey before moving into demographics. Respondents were asked to provide their role within the exhibition swine industry, such as parent, producer, or educator, as well as how long they had been involved with swine in any capacity. Both questions were free response to allow for a variety of roles and a wide range of time involved. Rather than asking about state of residence and states in which they exhibited swine, adults provided an average number of shows that their children or the youth they advise (if in an advising or mentoring role) attend per year. These questions were intended to help elucidate a relationship between length of time involved with exhibition swine and general knowledge of African Swine Fever and biosecurity, if one existed.

2.2.3 Survey Distribution

Both surveys were distributed between January 2022 and November 2022 through a variety of avenues. Livestock shows and jackpot circuits maintain email lists of exhibitors for registration purposes, and several participating shows agreed to distribute links via these lists. Posters were created advertising the surveys (appendix C) and displayed in prominent locations in the livestock barns at local county and state shows. The study team traveled to eight shows (Table 2.1) to distribute posters and collect responses on iPads (Apple Inc.; Cupertino, CA) at the county, state, and national level. Finally, a nationwide exhibition swine marketing firm (Showpig.com; Plain City, OH) was hired to send survey links via text messages and social media postings to their customer base in California, Georgia, Oklahoma, Pennsylvania, Tennessee, and Texas. Data collection ended on November 30th, 2022.

2.2.4 Survey Data Analysis

Survey responses were exported from Qualtrics to Microsoft Excel (Microsoft Corp.; Redmond, WA). All surveys under 10% complete were removed as this represented the individual moving past the first page with introductory information without answering any questions. Multiple choice questions were coded for correct responses and answers were aggregated to run basic analyses in Excel, including averages and percentages. Data were imported into a commercially available statistical analysis software (R version 4.2.2). Linear regressions and analyses of variance (ANOVA) were performed, and least square means were separated using an alpha of 0.05.

2.3 RESULTS

2.3.1 Youth Demographics

Following data cleaning, n = 127 usable youth survey responses were obtained. The majority of participants reported Colorado as their state of residence (72 responses, or 62.6%), and the next-largest group was nine participants from Oklahoma (7.8%). Other states reported were Texas, Florida, Ohio, Indiana, Pennsylvania, California, Nebraska, Georgia, Illinois, Michigan, Iowa, and Kentucky (Table 2.2). Participant age range, as shown in Figure 2.1, was 7 to 21 years old, with an average of 15.7 years. Participants indicated they had exhibited pigs for an average of six years and the oldest respondents reported showing pigs for nearly their entire lives, while some had not yet been involved for a full year (Figure 2.3).

Over half (59.4%) of respondents had exhibited pigs outside their state of residence within the past year. The top four states in which respondents reported exhibiting pigs were Colorado, Kentucky, Texas, and Nebraska. Michigan and Pennsylvania were marked as states of residence but not exhibition states. On average, participants had exhibited pigs in 2.5 states with a minimum of one and a maximum of 14 states (Figure 2.2). While many respondents (40.9%) were not members of any swine organization, 28.3% and 35.4% were members of a national or state organization, respectively (Table 2.3).

Among those who traveled out of state for shows, 76.8% attended non-terminal shows only, and 23.1% attended both terminal and non-terminal. These shows were primarily large national exhibitions, such as the National Western Stock Show or NJSA regional shows (40.1%) and state or local jackpot circuits like the Colorado Junior Swine Association series (39.3%), with a smaller number of respondents (20.4%) traveling for state or county fair open shows. Most youth who exhibited only in their state of residence attended both types (50%), but 38.6% attended non-terminal shows only and a small group (11.3%) attended terminal only. The majority of these shows were county fair or chapter shows (35.6%), local jackpot series (29.5%), and state fairs (27.8%), and a small number of respondents (6.9%) attended national shows in their home state.

2.3.2 Youth Perceptions and Understanding

Respondents answered a series of questions regarding biosecurity basics and details about ASF. Rating their general understanding of biosecurity principles from 1 (do not understand) to 5 (understand very well), youth averaged 3.5 and most frequently reported 5 (36 respondents) as shown in Figure 2.4, while their concern about ASF from 1 (not concerned) to 5 (very concerned) averaged 3.1 with a mode of 3 (44 respondents) as seen in Figure 2.5. When assessed in linear regression models, there was no relationship ($p > 0.05$) between biosecurity understanding and age, ASF concern and age, or ASF concern and years involved in the swine industry. The relationship between biosecurity understanding and years involved was statistically significant ($p < 0.05$), with each year of involvement adding approximately 0.09 units of understanding (correlation coefficient = 0.09002). There were no trends in one-way analyses of variance (ANOVAs) between biosecurity understanding and type of show attended or ASF concern and type of show attended, both in and out of state.

African Swine Fever has not been found in the United States. However, 46% of youth respondents said “yes” or “I don’t know” when asked if the virus has been detected in the U.S, and 90.5% said it can be transmitted to exhibition swine. As seen in Table 2.4, 75.4% correctly chose “infectious virus affecting wild and domestic pigs” as the answer to “What type of disease is ASF and what kinds of pigs does it affect?” Alternately, 21.3% responded that it is a bacterium and under 5% believed it could only affect wild pigs. When presented with four methods of disease spread and asked to select all that apply or none, if appropriate, respondents most frequently chose “direct contact with infected animals” (87.3%) and least frequently chose “ASF cannot spread between pigs” (7.9%). All four methods are possible for ASF transmission, and only 16.6% of respondents correctly selected all answers (Table 2.5).

The clinical presentation of ASF is like that of many other swine diseases including zoonoses, raising the importance of youth being able to recognize common symptoms to promote all facets of animal health. The following symptoms, all of which have been seen in clinical ASF cases, were listed for youth to choose from: high fever, lethargy, diarrhea, vomiting, coughing, decreased appetite, and red or blotchy skin. It was possible to select all answers or none of the above. Youth were readily able to identify high fever and diarrhea as symptoms, with 85.7% and 77.7% selecting these answers. Lethargy was the least-selected symptom (48.4%) and 26.9% of respondents correctly identified all seven. Merely 1.5% of youth could not identify any symptoms (Table 2.6).

Due to the likelihood of animals encountering diseases at exhibitions, it is recommended to quarantine animals away from the rest of the herd after returning home (National Association of State Public Health Veterinarians, 2018). Youth exhibiting outside of their home state were more likely to quarantine animals (53.7%) compared to those exhibiting only in their home state (43.1%). The percentage of exhibitors reporting that they had sick pigs after out of state shows (22%) was double that of in-state exhibitors (11%).

2.3.3 Adult Demographics

Following data cleaning, n = 211 usable adult survey responses were obtained. The respondents were asked to self-report their role(s) in the exhibition swine industry. A majority were parents of current exhibitors (42.32%) or showpig breeders (31.46%) at the time of data collection. The next-largest subset included those in a “fitter” role, meaning individuals who help prepare animals for competitions and may or may not also be parents of exhibitors (8.61%). Other roles reported, as shown in Table 2.7, were education, Extension, commercial industry, family other than parents, feed sales, animal health, and animal sales. Respondents could report more than one role, and 211 surveys yielded 267 roles, resulting in an average of 1.26 roles per person. This indicates that adults in the exhibition swine industry frequently hold more than one role within the niche, such as parent and breeder or Extension agent and feed representative.

When asked to indicate years of involvement in the swine industry, results ranged from under one year to 70 years (Figure 2.6). The mean was 21.74 years with a mode of 10 years, indicating that most respondents likely got their start in the industry as youth exhibitors and have stayed involved throughout their life. The largest portion of respondents (n = 68) reported 1-11 years involved, and 48 respondents reported 12-21 years. While only two respondents fell into the 62–71-year category, the inclusion of participants at almost every time point indicates the potential for lifelong involvement in the exhibition swine industry beyond the eligible ages for youth classes.

Adult respondents reported the number of exhibitions attended per year, on average, by their children or the youth they advise. The minimum reported was zero while the maximum was 200. Both the median and mode were 10 exhibitions with an average of 14.26, with many responses (n = 67) falling between 1 and 11 shows attended annually. This follows the pattern of previous studies on the showpig industry, where most exhibitors attend a small number of shows and only a tiny subset attends over 20.

2.3.4 Adult Perceptions and Understanding

Adult respondents were similarly asked to rate their understanding of biosecurity principles and concern surrounding ASF. The average biosecurity understanding rating (Figure 2.7) was 3.9 and 87 respondents chose 5, “understand very well.” ASF concern ratings (Figure 2.8) averaged 3.4, and an equal number of respondents (n = 61) chose 3 and 5, or “very concerned.” The increase in biosecurity understanding as an individual is involved in the swine industry for longer periods of time is significant ($p < 0.05$, correlation coefficient = 0.00503), as is the increase in ASF concern with years involved (correlation coefficient = 0.006046). No relationship exists between biosecurity understanding and number of shows attended by youth advised, although the relationship between ASF concern and shows attended is significant (correlation coefficient = 0.013106).

All adults chose at least one symptom of ASF and 32.7% of respondents correctly marked all listed symptoms (Table 2.8). Fever was the most frequently identified symptom, chosen by 92.4% of adults, and loss of appetite was chosen by 73.4% followed closely by lethargy at 72%. Most respondents, 89.1%, were aware that ASF is a virus infecting wild and domestic pigs and that it can spread to exhibition swine (94.7%). Although over half, 55.9%, knew that ASF has not been found in the U.S., a combined 44% selected “yes” or “I don’t know” in response to this question. ASF can spread in multiple ways, including via direct contact with infected animals, on shared equipment used on infected animals, through the air, or in feed. When offered these four choices, 23.2% of adults correctly chose all four (Table 2.9). Direct contact was most likely to be recognized as a mechanism of spread (90.5%) and feed was the least likely (36.4%), while 14.6% of respondents selected “ASF cannot spread between pigs.”

2.4 DISCUSSION

The results described above demonstrate a variety of levels of understanding in the realm of foreign animal diseases and biosecurity among youth swine exhibitors and the adults who parent and advise them on a day-to-day basis. Both groups are, in general, aware of biosecurity basics and general guidelines, although they may not follow such recommendations at shows. They know at least one symptom of common swine diseases and can identify several methods of disease spread. As expected, the significant correlation between biosecurity understanding and years involved for both groups, indicates that, like most activities, an individual’s knowledge increases proportionally to the time they have been involved. However, increased ASF concern correlated to more years involved in adults but not the youth surveyed. This may be due to a combination of factors, primarily that adults in the swine industry often work in and around multiple facets of the livestock and meat production industries, and thus are more likely to learn about foreign animal disease prevention and preparedness, including ASF.

Meanwhile, youth exhibitors may only be exposed to the swine industry through their show pigs, which is a self-insulating niche that does not provide much education on biosecurity and foreign animal diseases. This does not have to be the industry norm moving forward. Over 60% of the youth surveyed were members of a state, national, or local swine organization, providing an avenue for outreach and education on infectious diseases and biosecurity. Some of these organizations have begun working with university teams to offer such education in the trade show section of their exhibitions, often coupled with ongoing research projects in which youth are able to participate.

These results, particularly the ability of youth exhibitors to recognize common symptoms of disease while not knowing if ASF has been found in the U.S., are roughly in line with previous surveys of youth livestock exhibitors, in which Ohio swine exhibitors were most likely to follow the CDC recommendation to disinfect equipment before exhibitions (National Association of State Public Health Veterinarians, 2018), but 80% reported risky habits like eating and drinking in animal barns (Nolting et al., 2019). At the 2017 Kansas State Fair, 30% of swine exhibitors said they never disinfected shared equipment before using it on their animals (Larson, 2017). Prior to this study, little was known about adult knowledge and perceptions, specifically surrounding ASF and biosecurity. Adult agricultural educators reported a lack of knowledge on zoonoses and infectious diseases, but still believe they should be responsible for teaching these subjects to their students (Holub, 2017).

Youth exhibitors answered an open-ended question regarding biosecurity recommendations that they follow before, during, and after livestock shows, such as cleaning pens prior to moving animals in, using dividers between strange animals, and quarantining animals after the show. Most responses included the previous examples and additional precautions including not sharing equipment, cleaning and disinfecting all equipment before and after the show, and disinfecting their trailer after taking animals home. Some respondents indicated that they do not follow any biosecurity precautions. The results described above indicate widely varied levels of biosecurity understanding, and even individuals who claim to understand biosecurity principles very well may not recognize the importance of following these precautions at exhibitions to safeguard human and animal health.

Many diseases that animals may contract at exhibitions are not fatal and could remain subclinical for the duration of the infection, creating a challenge for those attempting to educate exhibitors and the adults involved in the industry about infectious diseases and biosecurity. It is remarkably difficult to emphasize the potential devastation that ASF, a disease that has never been found in the U.S., could cause. Given that the exhibition swine industry is a uniquely American niche, no other country’s outbreak in commercial animals, regardless of scale, is an appropriate analog to the possible situation in the event of ASF incursion into the U.S.

In conclusion, the discrepancies in knowledge, understanding, and perceptions of youth swine exhibitors and adults involved with exhibition swine represent a significant knowledge gap in foreign animal disease prevention and preparedness in the U.S. swine industry. As the country bolsters its defenses against ASF, it is increasingly important to address this gap. Although it is likely impossible to bring the exhibition swine industry up to the same biosecurity level as the commercial industry given its very nature, there are measures to be taken that will protect animal health, improve knowledge in both youth and adults, and ensure the U.S. swine industry can continue operating at its current capacity.

2.5 TABLES AND FIGURES

Table 2.1. 2022 exhibitions to which the study team traveled for in-person survey collection.

Exhibition	Location
National Western Stock Show	Denver, CO
CSU Main St. Mayhem Jackpot	Greeley, CO
Stan Brehon Jackpot	Greeley, CO
Phillips County Showdown Jackpot	Holyoke, CO
NJSA Summer Spectacular	Louisville, KY
Weld County Fair Swine Show	Greeley, CO
Larimer County Fair Swine Show	Loveland, CO
Colorado State Fair	Pueblo, CO

Table 2.2. Count and percent of reported states of residence for youth swine exhibitors (n = 115).

State	Count	Percent (%)
Colorado	72	62.61
Oklahoma	9	7.83
Texas	8	6.96
Florida	5	4.35
Ohio	5	4.35
Indiana	4	3.48
Pennsylvania	3	2.61
California	3	2.61
Nebraska	1	0.87
Georgia	1	0.87
Illinois	1	0.87
Michigan	1	0.87
Iowa	1	0.87
Kentucky	1	0.87

Table 2.3. Distribution of youth membership in national, state, or local swine organizations (n = 136).

Organization	Count	Percent (%)
National (NJSA/Team Purebred)	36	28.35
State (CJSA, etc.)	45	35.43
None	52	40.94
Local (4-H/FFA)	3	2.36

Table 2.4. Distribution of youth responses for pathogen type of ASF and variety of pigs infected.

Type of Pathogen	Count	Percent (%)
Infectious virus affecting wild and domestic pigs	95	75.40
Infectious virus affecting only wild pigs	4	3.17
Infectious bacteria affecting wild and domestic pigs	25	19.84
Infectious bacteria affecting only wild pigs	2	1.59

Table 2.5. Numbers and percentages of youth selecting one of four methods of ASF transmission (n = 115).

Method of spread	Count	Percent (%)
Direct contact with infected animals	110	87.30
Equipment shared with infected animals	90	71.43
Through the air	49	38.89
In feed	47	37.30
ASF cannot spread between pigs	10	7.94
All correct	21	16.67

Table 2.6. Numbers and percentages of youth correctly choosing each of seven symptoms provided in a list (n = 115).

Symptom	Count	Percent (%)
High fever	108	85.71
Lethargy	61	48.41
Diarrhea	98	77.78
Vomiting	67	53.17
Coughing	80	63.49
Decreased appetite	85	67.46
Red or blotchy skin	64	50.79
All correct	34	26.98
None correct	2	1.59

Table 2.7. Industry roles reported by adult survey respondents (n = 211).

Industry Role	Number	Percent (%)
Parent	113	42.32
Breeder	84	31.46
Fitter	23	8.61
Education	21	7.87
Extension	11	4.12
Commercial industry	6	2.25
Family	3	1.12
Feed sales	3	1.12
Animal health	2	0.75
Animal sales	1	0.37

Table 2.8. Distribution of adult respondent selection of seven symptoms provided in a list. (n = 211).

Symptom	Count	Percent (%)
High fever	195	92.42
Lethargy	152	72.04
Diarrhea	147	69.67
Vomiting	122	57.82
Cough	111	52.61
Loss of appetite	155	73.46
Red or blotchy skin	120	56.87
All correct	69	32.7

Table 2.9. Distribution of methods of ASF transmission chosen by adult respondents (n = 211).

Method of spread	Count	Percent (%)
Direct contact with infected animals	191	90.52
Equipment shared with infected animals	154	72.99
Through the air	94	44.55
In feed	77	36.49
ASF cannot spread between pigs	31	14.69
All correct	49	23.22

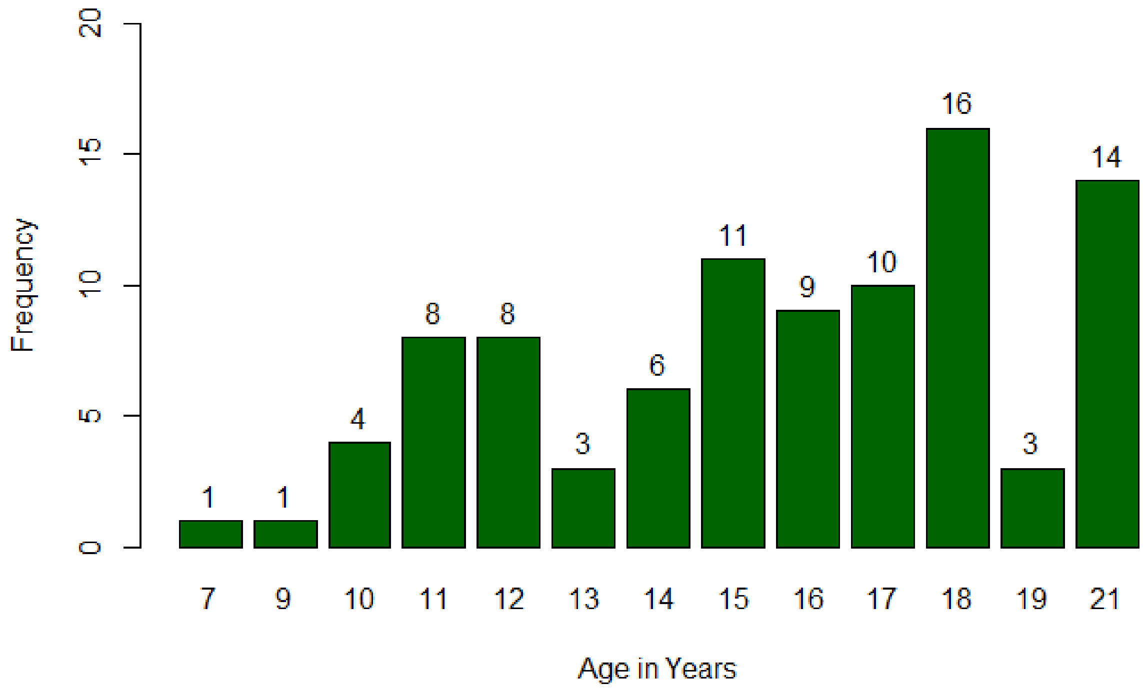

Figure 2.1. Bar chart of ages for youth swine exhibitors surveyed who opted to provide their age (n = 94).

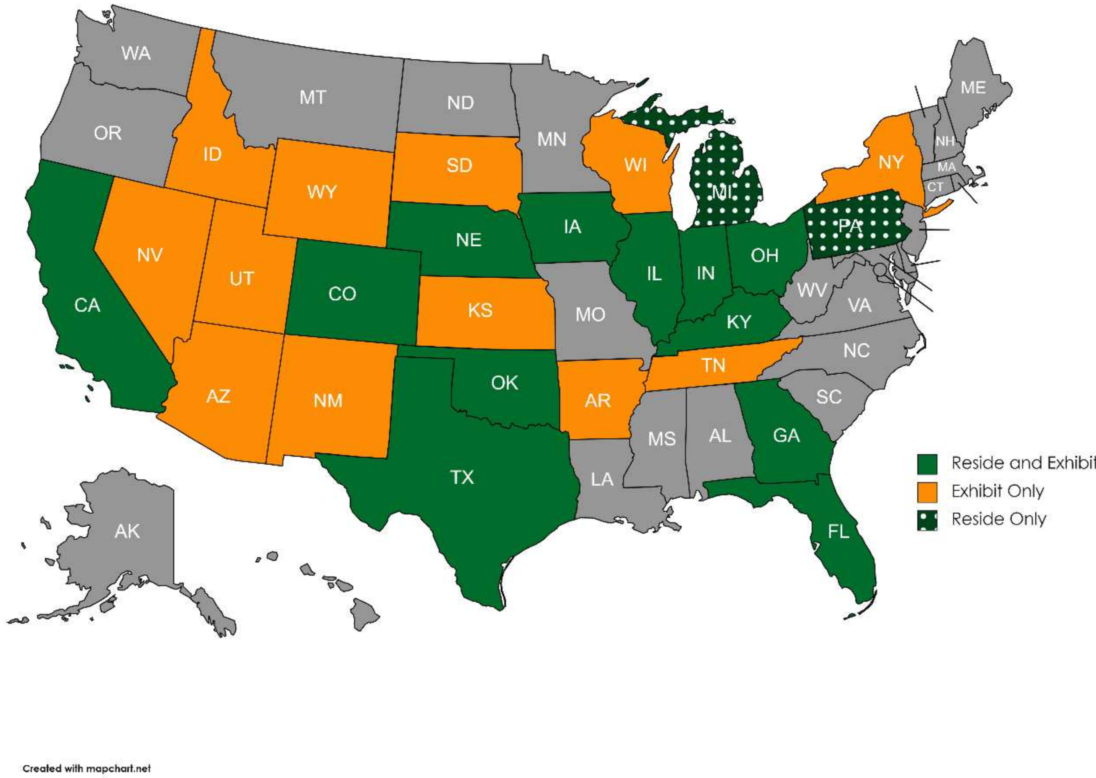

Figure 2.2. Map of states in which youth exhibitors reported both residing and exhibiting, residing only, and exhibiting only.

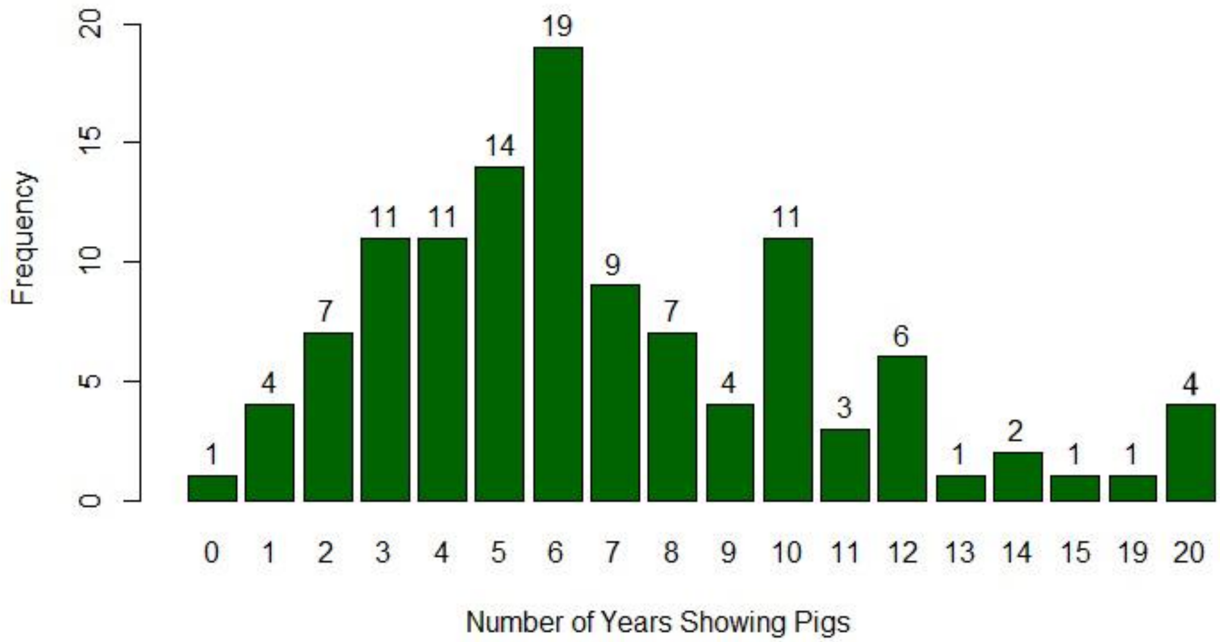

Figure 2.3. Bar chart showing the distribution of years showing pigs for exhibitors who provided this number (n = 117)

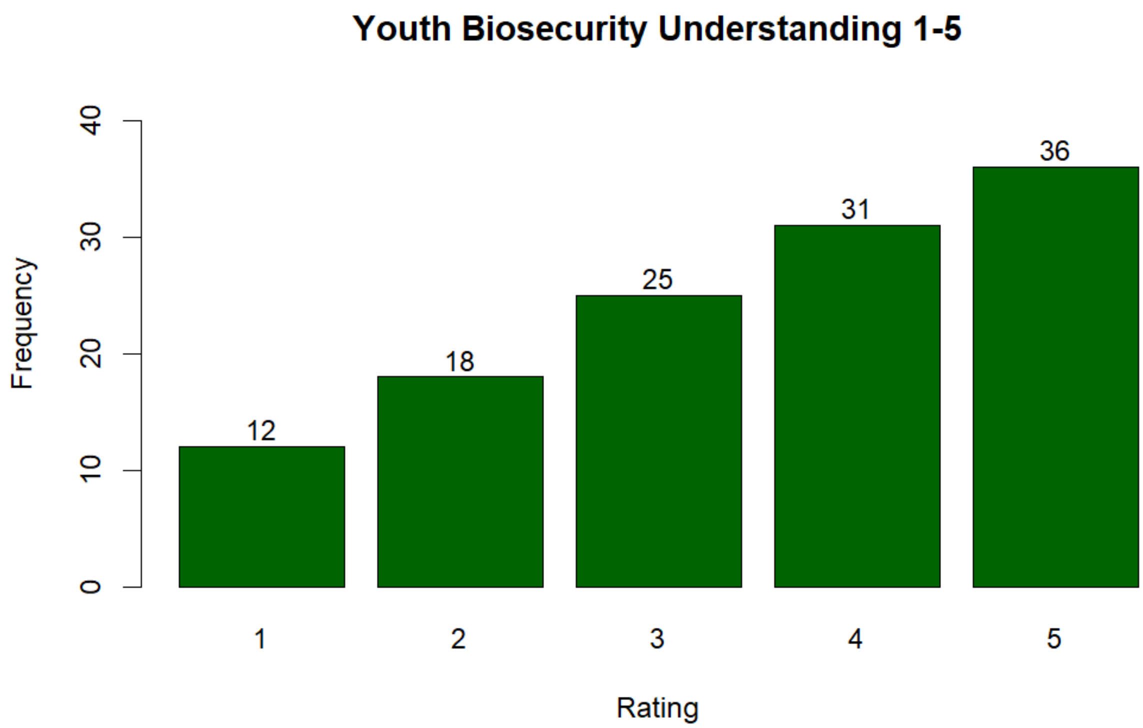

Figure 2.4. Youth self-evaluation of biosecurity understanding from 1 (do not understand) to 5 (understand very well).

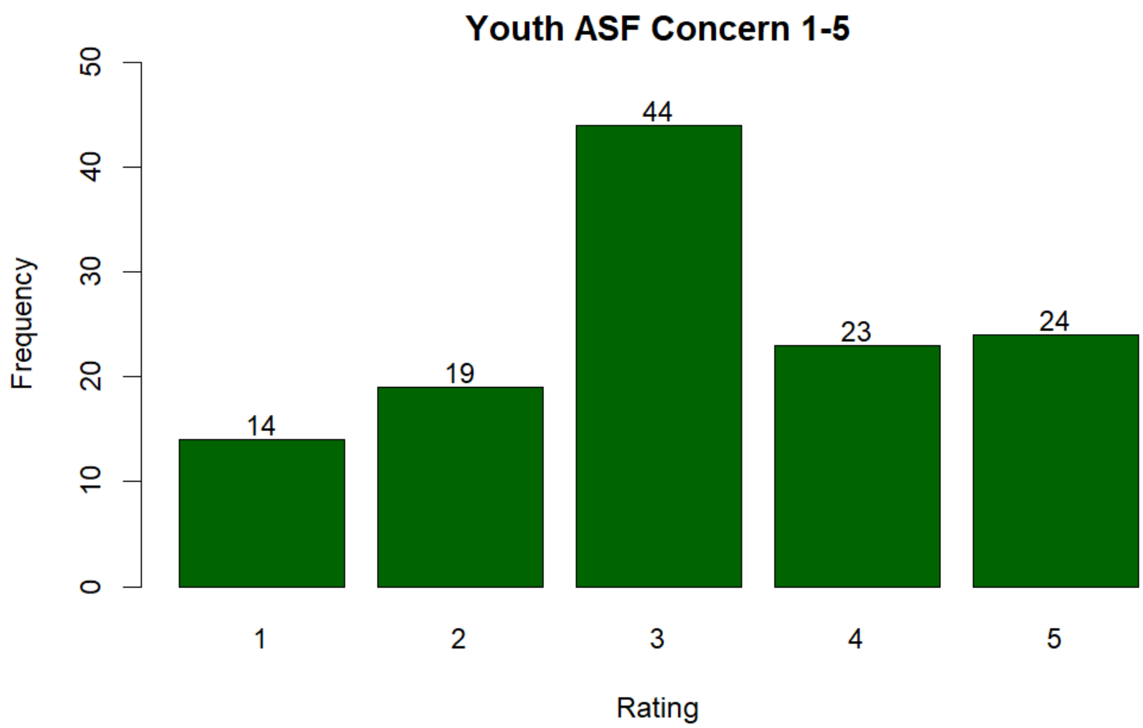

Figure 2.5. Youth self-evaluation of their concern surrounding ASF from 1 (not concerned) to 5 (very concerned).

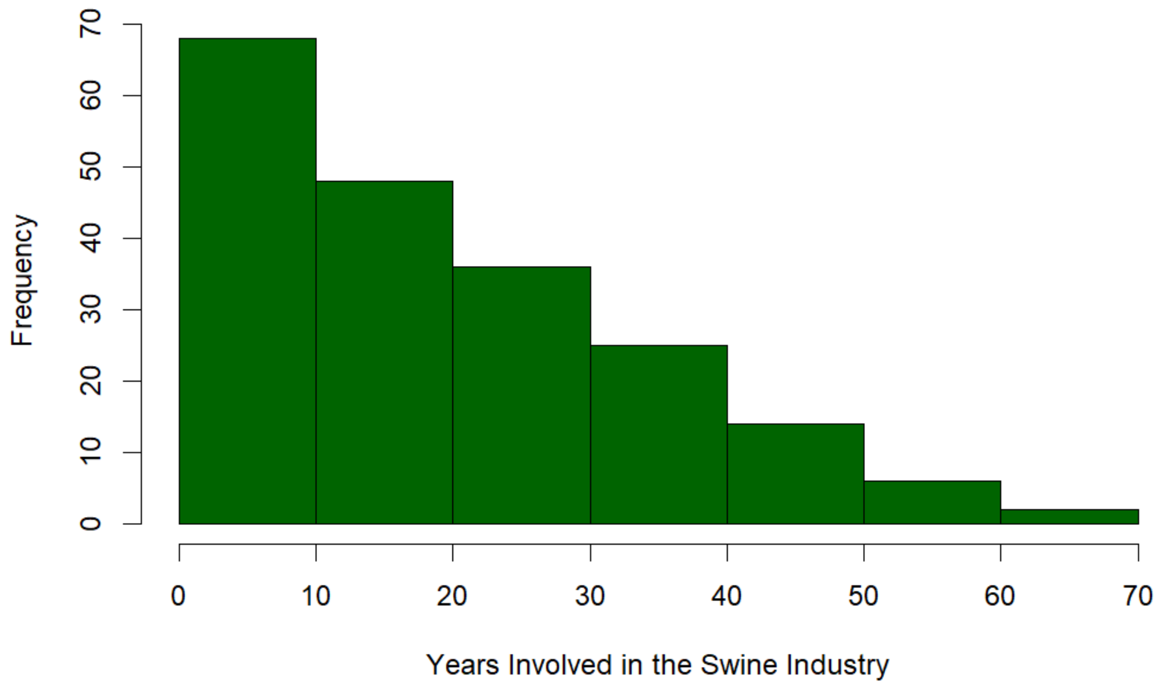

Figure 2.6. Histogram of years adult respondents have been involved in the swine industry in any capacity, binned by 10 years (n = 198).

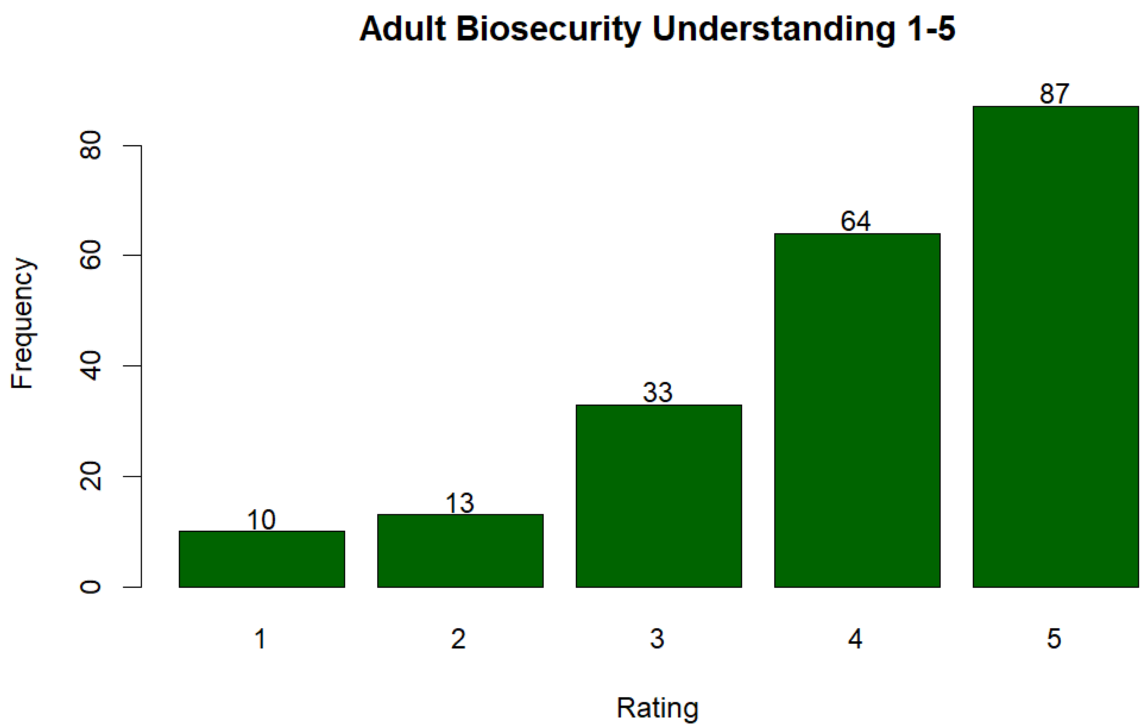

Figure 2.7. Adult respondent self-evaluation of their biosecurity understanding from 1 (do not understand) to 5 (understand very well).

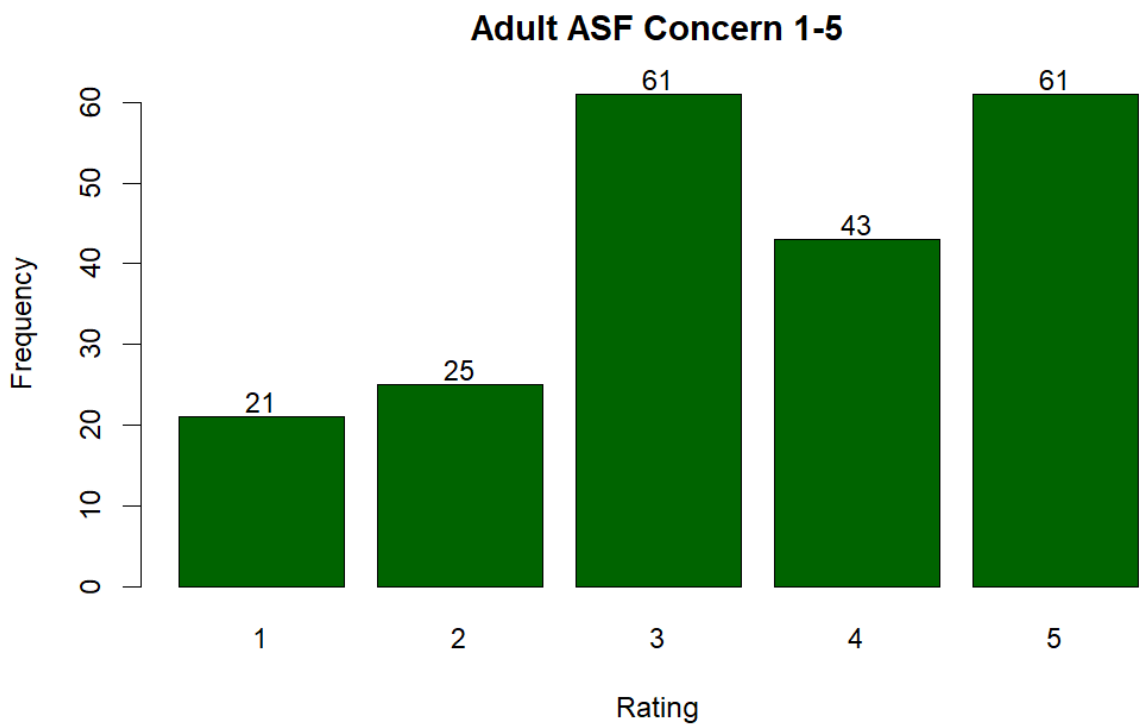

Figure 2.8. Adult respondent ASF concern from 1 (not concerned) to 5 (very concerned).

CHAPTER III: REPLACING A FRACTION OF LEAN BEEF WITH COLLAGEN POWDER IN BEEF FRANKFURTERS DOES NOT AFFECT SENSORY CHARACTERISTICS

3.1 INTRODUCTION

The simple hot dog is an American staple. Americans consume an estimated 20 billion hot dogs each year, according to the National Hot Dog and Sausage Council (Hot Dog Consumption Statistics). United States code of federal regulations defines frankfurters, franks, hot dogs, and similar products as comminuted sausages prepared from one or more kinds of skeletal muscle meat that must be seasoned and cured (9 CFR 319.180). Preference for protein type varies by region and age of the consumer, but most commercially available brands consist of beef, pork, poultry, or some combination thereof. The COVID-19 pandemic shocked meat supply chains, resulting in higher purchase prices for consumers before the markets began returning to their pre-shock patterns in roughly 4-6 months (Erol and Saghaian, 2022). Such supply chain impacts emphasize the importance of incorporating all edible proteins from beef carcasses, such as collagen, to reduce costs. Collagen is a protein found in many body tissues including some of the byproducts removed during the slaughter process like bones and tendons. All byproducts, edible and inedible, add roughly $182 in value to the average fed steer carcass, according to the USDA Agricultural Marketing Service. Novaprom, a business unit of the JBS Group, produces natural dehydrated beef stock (NDBS) collagen powder under the commercial name NOVAPRO®. Through in-house testing, Novaprom saw increased water holding capacity, shelf life, and emulsion stability in some processed meat products, including sliced and ground and formed items, although it was previously untested in beef frankfurters. This study incorporated NDBS into beef frankfurters at three hydration levels (4:1, 5:1, and 6:1

water:NDBS) and evaluated objective and subjective sensory characteristics, along with cook yield, water activity, and pH.

3.2 MATERIALS AND METHODS

3.2.1 Beef Frank Production

Prior to production, raw materials (beef 50/50: 8/12/2021, lot 247869, EST 19652) and 90/10 trim: 3/23/2022, lot 266405, EST 969) were thawed in a 0°C cooler for three days to final temperatures of 1.1°C and 0°C, respectively. The composition of each raw material source is found in Table 3.1. NOVAPRO® NDBS (Novaprom; São Paulo, Brazil) was received and stored at room temperature until use.

Beef franks were produced according to a standard hot dog processing protocol in 25 lb. batches for each of three test formulations (Table 3.2) and the control. Lean beef was ground in an industrial mixer/mincer (Thompson 4000; Crestmead QLD, Australia) through a 1/8" plate and the fat component was ground through a 3/8" plate before being transferred to a vacuum bowl chopper (Seydelmann K 60; Stuttgart, Germany) for one minute. Sodium phosphate (Walton's; Wichita, KS) was added to the batter and chopped for 30 seconds. Test formulations were produced by adding 0.2 lb. NDBS powder and 0.8 lb., 1 lb., or 1.2 lb. of water for the 4:1, 5:1, and 6:1 formulas, respectively. All batches included 2/3 lb. water, 2 lbs. hot dog seasoning (Walton's; Witchita, KS), and 1 oz. Heller's modern cure salt 6.25% (Newly Weds Foods; Chicago, IL) and were chopped for one minute after the addition of these ingredients. The fat component was added and chopped until it blended into the batter, then the product was transferred to an industrial stuffer (Handtmann VF628; Lake Forest, IL), linked into sausage casings, and weighed in treatment batches on a scale tared with smoke sticks.

Beef franks were then smoked in a commercial smokehouse (Enviro-Pak; Clackamas, OR) to a core temperature of 68.3°C with humidity varying by cycle according to the cook schedule in Table 3.3. After smoking, franks were weighed again, peeled out of casings, and vacuum sealed in packages of 10, then boxed separately according to treatment and stored in a 0° cooler until evaluation.

3.2.2 Evaluation of Beef Franks

Beef franks were held in storage for 3-5 days until tests were performed. Ten franks from each treatment were allocated to shear force. Ten grams of product were removed from each of 5 franks, chopped, and homogenized for a total of 50 grams per treatment, then the homogenate was used for pH analysis in triplicate and water activity testing in duplicate. Approval was obtained from the Colorado State University IRB for protocol number 3501, and ten franks per treatment were allocated for sensory panels.

3.2.3 Warner-Bratzler Shear Force Testing

Franks were held at room temperature for Warner-Bratzler shear force testing protocols recommended by the 2016 American Meat Science Association (AMSA) Sensory Guidelines as described by Wheeler (Wheeler et al., 1996). This analysis was performed on a Universal Testing Machine (Instron Corp., Canton, MA) equipped with a flat, blunt-end blade (500mm/min crosshead speed and 100kg load capacity) intended to mimic the human bite force. Each sample was cut vertically into six sections and sheared once. The values were recorded and averaged into a single value for statistical analysis.

3.2.4 pH and Water Activity Testing

Ten grams of sample from each treatment were vortexed with 90 mL of distilled water and the pH was measured using an appropriately calibrated pH meter (ThermoScientific Orion Star A121; Waltham, MA). Two replicates were performed to obtain a total of three pH values per treatment. Two samples were evaluated for each treatment to determine water activity measurements on a commercial water activity meter (AquaLab Model Series 4; Pullman, WA).

3.2.5 Trained Sensory Evaluation

Panelists were trained to evaluate attributes with methods and anchors from *Sensory Evaluation Techniques* by Meilgaard, Civille, and Carr (Meilgaard et al., 2016). During each training session, panelists were offered anchors to establish standards for each new attribute being introduced at that time and were then asked to evaluate commercially available hot dog samples to calibrate anchors on the appropriate product. Over the duration of 3 panels, trained sensory panelists (n = 5) evaluated 8 samples on a 0-15 scale (see ballot in Appendix D) for red intensity, beef flavor intensity, seasoning intensity, springiness, cohesiveness, mouth coating, off-odors, and off-flavors to obtain a total of 30 observations per treatment.

3.2.6 Statistical Analysis

All data were imported into a commercially available statistical analysis program (R). An analysis of variance (ANOVA) was performed for each characteristic and least square means were separated using an alpha of 0.05.

3.3 RESULTS

Cook yield (Table 3.4) was calculated for each treatment using the formula $(cooked\ weight - raw\ weight) * 100$. Regarding pH of cooked samples, there were no significant differences in pH among samples containing NBDS, which were all greater than the control samples (Table 3.5). As shown in Table 3.6, the shear force values of control samples were greater than all other formulations ($p<0.05$). No difference in water activity (A_W) was observed among treatment groups (Table 3.6). Among samples containing NBDS, the 4:1 formulation had a greater average shear force than either 5:1 or 6:1 formulations, which were both lower than the 4:1 formulation. Analysis of sensory characteristics and composition of the control samples compared to those containing NBDS revealed no statistical differences in any attributes between the four formulations (Figures 3.1 through 3.5).

3.4 DISCUSSION

The inclusion of collagen powder in processed meat products is a small effort to maximize the utility of beef cattle in the U.S. This study found no differences in trained sensory panels and there was a significant reduction in shear force values compared to the control, indicating a lower bite force required to chew the product. There was no significant difference in protein content between any of the treatments, thus the differences in shear force may be attributed to the increase in fat content seen in the 5:1 and 6:1 batches. Greater fat content in frankfurters may correlate to lower shear force values (Matulis et al., 1994). Depending on which component of the frankfurter is replaced with an alternative protein like collagen, the products could contain up to 50% less fat while maintaining quality and improving firmness (Choe et al., 2013).

Additional research is recommended to evaluate the reception of treatment batches by consumer panels when compared to commercially available beef frankfurters, but the lack of statistical differences between hydration levels is promising for promotion of the NOVAPRO® product to further processing companies. A 2017 study found that hydrolyzed collagen improved the texture and increased water holding capacity of beef and pork frankfurters when replacing up to 50% of the pork fat component without sacrificing product quality (Sousa et al., 2017). Future directions could address multiple protein types incorporated into one frankfurter with NDBS powder replacing a fraction of the protein or fat to evaluate the effect on sensory characteristics and composition of mixed-protein franks.

In conclusion, replacing a fraction of lean beef with collagen powder in frankfurters did not significantly affect trained sensory evaluation, although objective shear force values decreased at all three hydration levels. Trained panelists did not detect differences between the four batches presented, but consumer perception was not evaluated. The lack of significant differences observed in this study suggests the ability to market this product to further processing companies as an option to lower costs and reduce shear force, pending regulatory approval.

3.5 FINAL SUMMARY

Foreign animal diseases remain a real threat to food supply chains in the U.S. The best efforts to protect market access for animal proteins are multifaceted, and should incorporate all aspects of the meat industry. Ensuring swine producers are following operational biosecurity protocols, assessing vulnerabilities in the exhibition swine industry, and developing robust biosecurity and foreign animal disease educational resources for all ages are critical measures to safeguard continuity of business. Given that a majority of youth exhibitors and the adults who mentor them can be reached through local, state, and national swine organizations, education efforts should be focused on these groups. Finally, processed meat producers should investigate their ability to improve functionality of their products and lower costs with alternative proteins, such as collagen. These substitutions can help maximize carcass utility in the event of a foreign animal disease incursion to mitigate impacts of a loss of animals in the meat supply chain.

3.5 TABLES AND FIGURES

Table 3.1. Beef frank raw material composition expressed as percentages of protein, fat, moisture, and collagen.

Component	Protein (%)	Fat (%)	Moisture (%)	Collagen (%)
Lean (beef 90/10)[1]	19.74	8.29	68.16	2.50
Fat (beef 50/50)[1]	9.13	48.71	37.64	3.03

[1]Ratio of lean beef:fat contained in the raw materials.

Table 3.2. Formulations & cost analysis**.**

Treatment	**Ingredient (lbs.)**				**Cost Analysis ($/lb)**[1]	Cost Reduction[2]
	Lean	50/50 trim	NDBS	Hydration Water		
Control	12	13	0	0.00	49.12	-
4:1[3]	10.37	13.28	0.27	1.08	46.74	4.85%
5:1[3]	10.03	13.35	0.27	1.35	45.89	6.58%
6:1[3]	9.71	13.40	0.27	1.62	45.07	8.24%

[1]Cost analysis was performed assuming the following variables:

- Lean Trim: $2.75/lb
- Fat Trim: $1.24/lb
- Water: $0.00/lb
- NBDS: $6.50/lb

[2]Cost reduction calculated as a percentage reduction from Control.
[3]Indicates ratio of water:NDBS powder by weight in each treatment.

Table 3.3. Smokehouse cook schedule followed for beef frank production.

Stage	**Time (min)**	**Core Temp (ºC)**	**Humidity (%)**
Dry	30	-	0
Smoke	60	-	30
Lethality	to temp	68.3	60
Shower	20	-	-

Table 3.4. Cook yield for each of four treatments.

Formulation	Raw (lb.)	Cooked (lb.)	Yield (%)
Control	18.6	16.85	90.59
4:1[1]	12.45	11.4	91.57
5:1[1]	21.1	19.45	92.18
6:1[1]	21.4	19.55	91.36

[1]Indicates ratio of water:NDBS powder by weight in each treatment.

Table 3.5. pH measurements for all samples collected.

Sample	Raw pH	Cooked pH
Lean trim	5.92	-
Fat trim	6.02	-
Control	6.23	6.26[a]
4:1[1]	6.21	6.33[b]
5:1[1]	6.23	6.31[b]
6:1[1]	6.26	6.31[b]

[a,b]Values with different superscripts are different ($p < 0.05$).

[1]Indicates ratio of water:NDBS powder by weight in each treatment.

Table 3.6. Water activity, shear force value, and composition (g/100g) for each treatment.

Sample	A_w	Shear force (kg)	Protein	Fat	Moisture	Collagen
Control	0.944[a]	1.37[a]	12.80 [a]	26.47[a]	52.34[ab]	3.16[a]
4:1[1]	0.943[a]	1.00[b]	13.08 [a]	26.31[a]	53.78[b]	3.28[a]
5:1[1]	0.946[a]	0.92[c]	13.30 [a]	28.05[b]	52.67[ab]	3.40[a]
6:1[1]	0.947[a]	0.89[c]	12.47 [a]	28.09[b]	51.95[a]	3.22[a]
SEM	0.0017	0.0152	0.55	0.78	0.18	0.28

[a,b,c] Values with different superscripts are different ($p < 0.05$).

[1]Indicates ratio of water:NDBS powder by weight in each treatment.

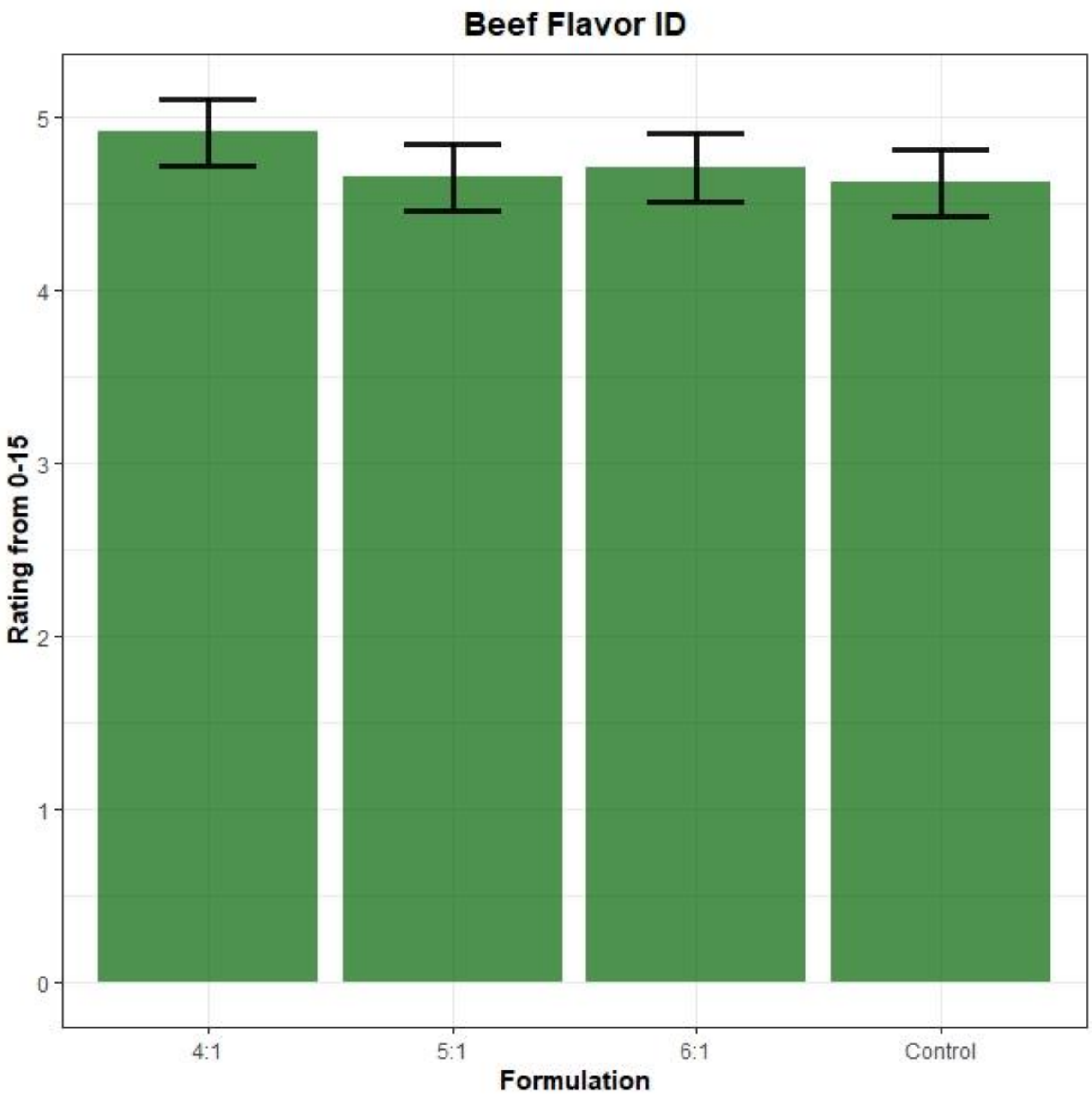

Figure 3.1. Trained panel data for beef flavor ID (p > 0.776, SE = 0.196).

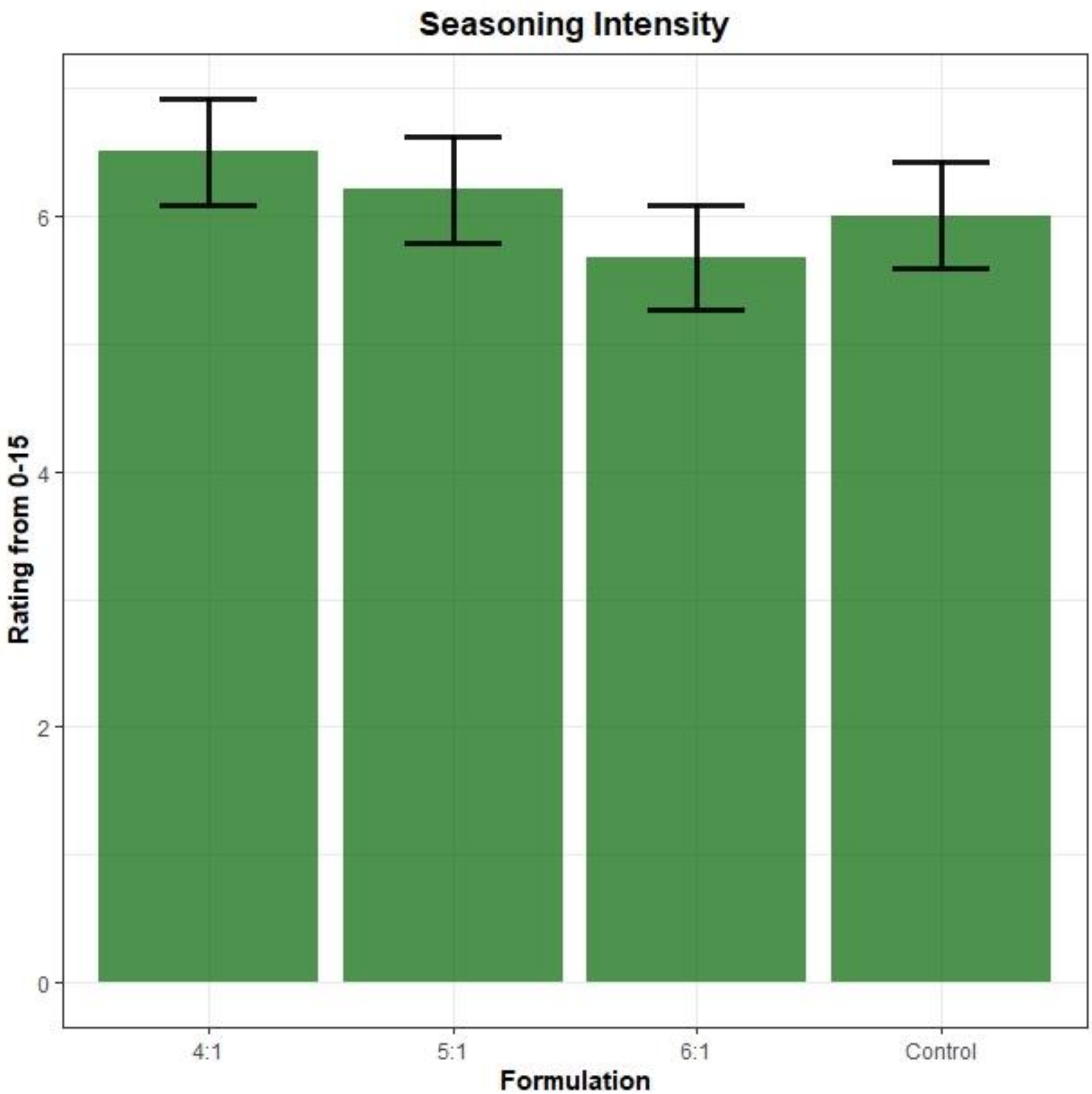

Figure 3.2. Trained panel data for seasoning intensity (p > 0.498, SE = 0.415).

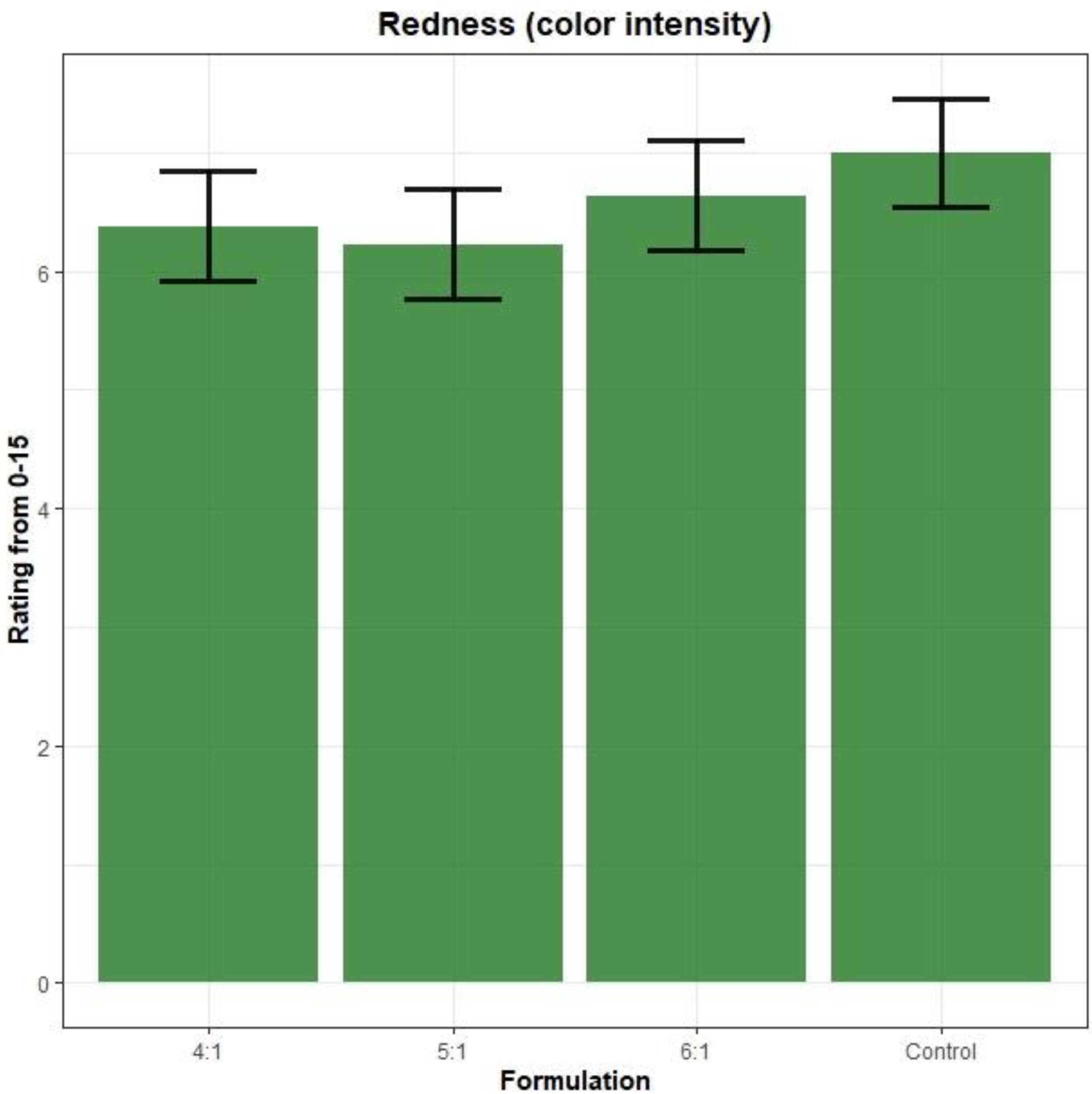

Figure 3.3. Trained panel data for redness (color intensity, $p > 0.642$, SE = 0.459).

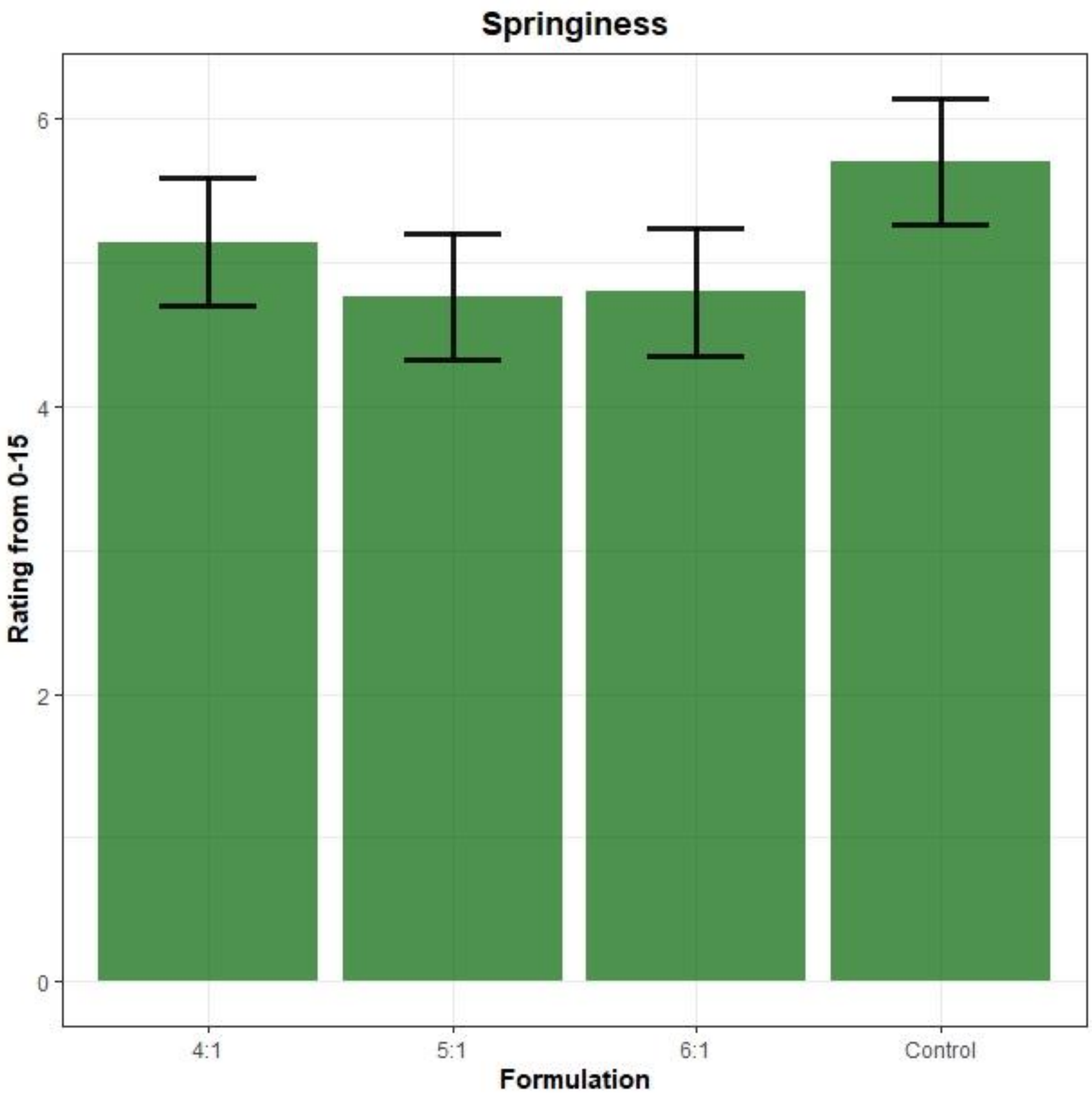

Figure 3.4. Trained panel data for springiness ($p > 0.145$, SE = 0.439).

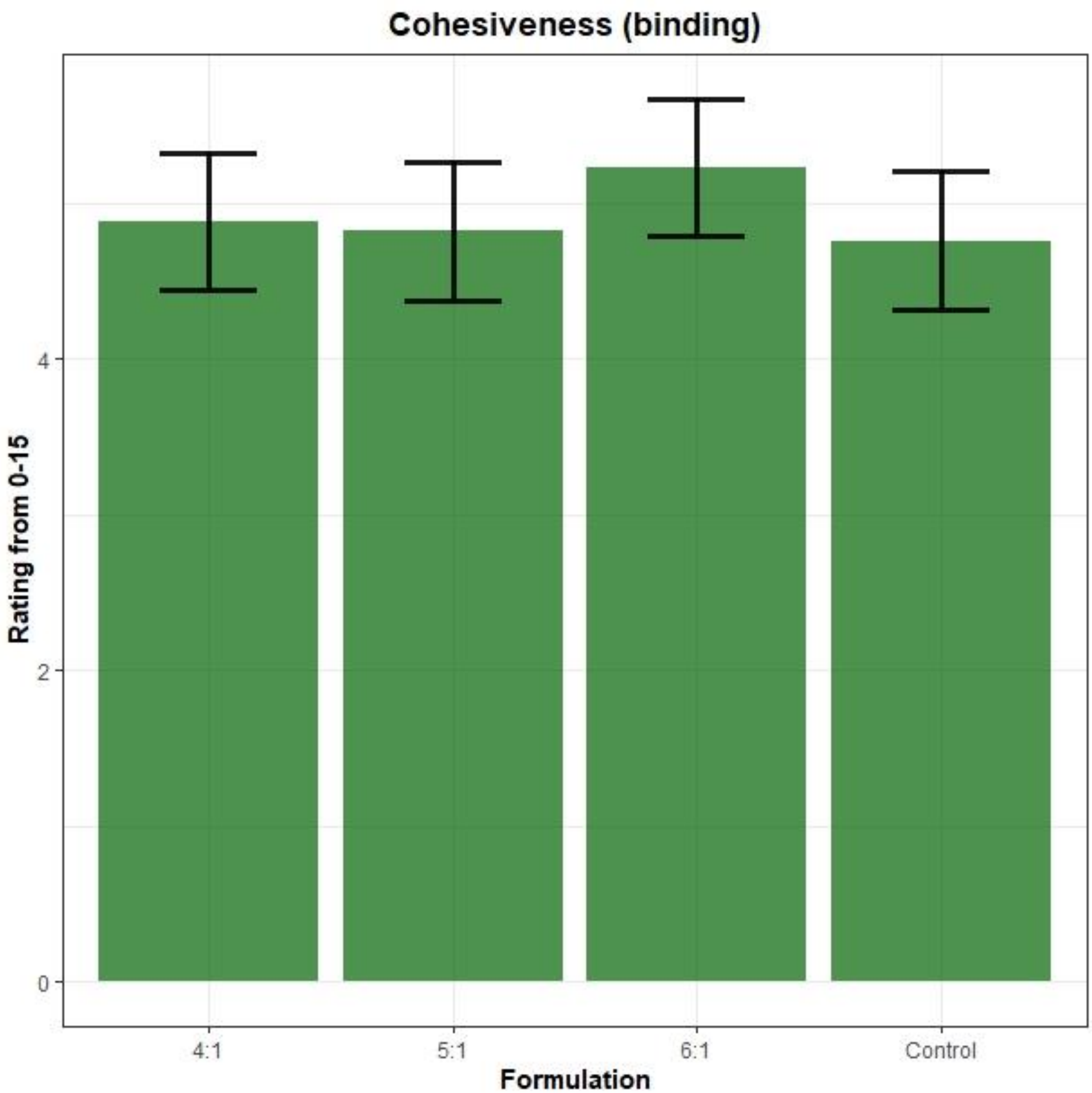

Figure 3.5. Trained panel data for cohesiveness (binding, $p > 0.712$, SE = 0.442).

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APPENDIX A: YOUTH SURVEY INSTRUMENT

Youth ASF Perceptions - Expanded

Start of Block: Introduction

Q1

Q2 This survey from the Colorado State University Department of Animal Sciences is intended to assess perceptions of African Swine Fever (ASF) among youth swine exhibitors. It will take approximately 5 minutes to complete. All responses will remain anonymous and will help inform best practices for youth biosecurity education and ASF prevention in the exhibition swine industry. Contact hannah.cochran@colostate.edu with questions.

End of Block: Introduction

Start of Block: Block 3

Q13 Are you over the age of 18?

- ○ Yes (1)
- ○ No (2)

Skip To: End of Block If Are you over the age of 18? = Yes

Skip To: Q14 If Are you over the age of 18? = No

Q14 If you are not over the age of 18, do you have permission from your parent/guardian to complete this anonymous survey?

◯ Yes (1)

◯ No (2)

◯ Unsure (3)

Skip To: End of Block If If you are not over the age of 18, do you have permission from your parent/guardian to complete t... = Yes

Skip To: Q15 If If you are not over the age of 18, do you have permission from your parent/guardian to complete t... = No

Skip To: Q15 If If you are not over the age of 18, do you have permission from your parent/guardian to complete t... = Unsure

Q15 Parent/guardian permission is required before completing the survey. Please obtain their permission before accessing the survey.

Skip To: End of Survey If Parent/guardian permission is required before completing the survey. Please obtain their permissi... Is Displayed

End of Block: Block 3

Start of Block: Perception Questions

Q3 What is African Swine Fever?

◯ Infectious virus affecting wild and domestic pigs (1)

◯ Infectious bacteria affecting wild and domestic pigs (2)

◯ Infectious virus affecting only wild pigs (3)

◯ Infectious bacteria affecting only wild pigs (4)

Q4 Can African Swine Fever be transmitted to show pigs?

- ☐ Yes (6)
- ☐ Maybe (7)
- ☐ No (8)

Q5 Has African Swine Fever been found in the United States?

- ○ Yes (1)
- ○ No (2)
- ○ I don't know (3)

Q7 What are the symptoms of African Swine Fever? Select all that apply.

- ☐ High fever (1)
- ☐ Lethargy (2)
- ☐ Diarrhea (3)
- ☐ Vomiting (4)
- ☐ Coughing (5)
- ☐ Decreased appetite (6)
- ☐ Red or blotchy skin (7)

Q8 How is African Swine Fever spread? Select all that apply.

- ☐ Direct contact with infected pigs (1)
- ☐ Equipment used on infected pigs (2)
- ☐ Through the air (3)
- ☐ It can't be spread between pigs (4)
- ☐ Feed (5)

Page Break

Q6 On a scale from 1 to 5, how concerned are you about African Swine Fever?

	1: Not concerned		5: Extremely concerned		Not Applicable
	1	2	3	4	5
Slide from 1-5. ()					

Page Break

Q9 On a scale from 1 to 5, rank your understanding of biosecurity principles.

	1: Do not understand		5: Understand very well		Not Applicable
	1	2	3	4	5
Slide from 1 to 5. ()					

End of Block: Perception Questions

Start of Block: Demographic questions

Q10 How many years have you shown pigs?

____

Q11 How old are you?

▼ 3 (4) ... 21 (22)

Q12 What, if any, swine organizations are you a member of? Select all that apply.

- ▢ National association (i.e. NJSA or Team Purebred) (1)
- ▢ State-based swine association (i.e. Colorado Junior Swine Association, etc.) (2)
- ▢ None (3)
- ▢ Other (please list) (4) ____

state In which state do you currently reside?

▼ Alabama (1) ... I do not reside in the United States (53)

End of Block: Demographic questions

Start of Block: Block 4

Q19 Have you traveled out of your home state for a swine show within the last year?

- ○ Yes (1)
- ○ No (2)

Skip To: Q25 If Have you traveled out of your home state for a swine show within the last year? = Yes

Skip To: End of Block If Have you traveled out of your home state for a swine show within the last year? = No

Page Break

Q25 If yes, what type of show?

- ○ Terminal (you did not bring pigs home) (1)
- ○ Non-terminal (you brought pigs home) (2)
- ○ Both (3)

Q20 Please indicate the level of show:

- ☐ National show (such as the National Western Stock Show or an NJSA Regional Show) (1)
- ☐ Jackpot (state or local) (2)
- ☐ State or county fair (open shows) (3)
- ☐ Other (please provide answer) (4) ____________________

State Which states did you visit for swine shows? Select all that apply.

- ▢ Alabama (1)
- ▢ Alaska (2)
- ▢ Arizona (3)
- ▢ Arkansas (4)
- ▢ California (5)
- ▢ Colorado (6)
- ▢ Connecticut (7)
- ▢ Delaware (8)
- ▢ District of Columbia (9)
- ▢ Florida (10)
- ▢ Georgia (11)
- ▢ Hawaii (12)
- ▢ Idaho (13)
- ▢ Illinois (14)
- ▢ Indiana (15)
- ▢ Iowa (16)

- ☐ Kansas (17)
- ☐ Kentucky (18)
- ☐ Louisiana (19)
- ☐ Maine (20)
- ☐ Maryland (21)
- ☐ Massachusetts (22)
- ☐ Michigan (23)
- ☐ Minnesota (24)
- ☐ Mississippi (25)
- ☐ Missouri (26)
- ☐ Montana (27)
- ☐ Nebraska (28)
- ☐ Nevada (29)
- ☐ New Hampshire (30)
- ☐ New Jersey (31)
- ☐ New Mexico (32)
- ☐ New York (33)

- North Carolina (34)
- North Dakota (35)
- Ohio (36)
- Oklahoma (37)
- Oregon (38)
- Pennsylvania (39)
- Rhode Island (41)
- South Carolina (42)
- South Dakota (43)
- Tennessee (44)
- Texas (45)
- Utah (46)
- Vermont (47)
- Virginia (48)
- Washington (49)
- West Virginia (50)
- Wisconsin (51)

☐ Wyoming (52)

Page Break

Q21 Which (if any) biosecurity recommendations did you follow at these shows? Examples include not sharing equipment, disinfecting pens, using dividers between strange animals.

____

Q22 Did you place animals under quarantine after returning from these shows?

○ Yes (1)

○ No (2)

○ Unsure (3)

Q23 Were any of your animals sick at these shows or shortly after coming home?

○ Yes (1)

○ No (2)

○ Unsure (3)

Skip To: End of Survey If Were any of your animals sick at these shows or shortly after coming home? , Yes Is Displayed

End of Block: Block 4

Start of Block: Block 5

Q26 If no, which type(s) of shows did you attend in-state?

- ○ Terminal (you did not bring pigs home) (1)
- ○ Non-terminal (you brought pigs home) (2)
- ○ Both (3)

Q27 Please indicate the levels of shows you attended within your home state:

- ☐ National show (such as the National Western Stock Show or NJSA Regional Shows) (5)
- ☐ State fair (1)
- ☐ Local jackpots (3)
- ☐ County fair or chapter show (4)
- ☐ Other (please provide answer) (6) ____

Q28 Which (if any) biosecurity recommendations did you follow at these shows? Examples include not sharing equipment, disinfecting pens, using dividers between strange animals.

____

Q29 Did you place animals under quarantine after returning from these shows?

- ○ Yes (1)
- ○ No (2)
- ○ Unsure (3)

Q30 Were any of your animals sick at these shows or shortly after coming home?

- ○ Yes (1)
- ○ No (2)
- ○ Unsure (3)

End of Block: Block 5

APPENDIX B: ADULT SURVEY INSTRUMENT

Adult ASF Perceptions

Start of Block: Introduction

Q1

Q2 This survey from the Colorado State University Department of Animal Sciences is intended to assess perceptions of African Swine Fever (ASF) among adults involved in the swine industry (such as parents/guardians, breeders, fitters, and educators). It will take approximately 5 minutes to complete. All responses will remain anonymous and will help inform best practices for youth biosecurity education and ASF prevention in the exhibition swine industry. Contact hannah.cochran@colostate.edu with questions.

End of Block: Introduction

Start of Block: Perception Questions

Q3 What is African Swine Fever?

- ○ Infectious virus affecting wild and domestic pigs (1)
- ○ Infectious bacteria affecting wild and domestic pigs (2)
- ○ Infectious virus affecting only wild pigs (3)
- ○ Infectious bacteria affecting only wild pigs (4)
- ○ Other (5) ____

Q4 Can African Swine Fever be transmitted to show pigs?

- ○ Yes (3)
- ○ No (4)
- ○ I don't know (5)

Q5 Has African Swine Fever been found in the United States?

- ○ Yes (1)
- ○ No (2)
- ○ I don't know (3)

Q7 What are the symptoms of African Swine Fever? Select all that apply.

- ▢ High fever (1)
- ▢ Lethargy (2)
- ▢ Diarrhea (3)
- ▢ Vomiting (4)
- ▢ Coughing (5)
- ▢ Decreased appetite (6)
- ▢ Red or blotchy skin (7)

Q8 How is African Swine Fever spread? Select all that apply.

- ▢ Direct contact with infected pigs (1)
- ▢ Equipment used on infected pigs (2)
- ▢ Through the air (3)
- ▢ It can't be spread between pigs (4)
- ▢ Feed (5)

Q6 On a scale from 1 to 5, how concerned are you about African Swine Fever?

	1: Not concerned				5: Very concerned
	1	2	3	4	5
Slide from 1-5. ()					

Page Break

Q9 On a scale from 1 to 5, rank your understanding of biosecurity principles.

	1: Do not understand				5: Understand very well
	1	2	3	4	5
Slide from 1 to 5. ()					

End of Block: Perception Questions

Start of Block: Demographic questions

Q13 What is your role within the swine industry (ex: parent/guardian, breeder, fitter, educator, extension agent)?

____

Q10 How many years have you been involved with swine?

____

Q12 How many swine exhibitions (i.e county fairs, state fairs, jackpots, national exhibitions, etc) do you or the youth you advise attend per year, on average?

____

End of Block: Demographic questions

Start of Block: Block 3

Q14 The US Swine Health Improvement Plan (SHIP) is launching in Colorado soon. This program enrolls producers and packers to collect biosecurity & movement information as a foreign animal disease prevention & preparedness measure with the potential for on-farm surveillance sample collection in the future. If you are interested in enrolling, please contact animalsciences@colostate.edu. More information can be found here: https://usswinehealthimprovementplan.com/

End of Block: Block 3

APPENDIX C: SURVEY ADVERTISEMENT MATERIALS

DO YOU WORK WITH SHOW PIGS?

Take this anonymous survey about African Swine Fever.

Point your phone camera at the QR code below.

ANIMAL SCIENCES
COLORADO STATE UNIVERSITY

CSU researchers are conducting a survey to assess adult perceptions of ASF. All responses are anonymous. Contact hannah.cochran@colostate.edu with questions.

DO YOU SHOW PIGS?

Take this anonymous survey about African Swine Fever.

Point your phone camera at the QR code below.

ANIMAL SCIENCES
COLORADO STATE UNIVERSITY

CSU researchers are conducting a survey to assess youth perceptions of ASF. All responses are anonymous. Contact jennifer.martin@colostate.edu with questions.

APPENDIX D: BEEF FRANK TRAINED SENSORY PANEL BALLOT

Hot Dog Trained Sensory Panel #1

Start of Block: Initials

Q23 Panelist initials

____

End of Block: Initials

Start of Block: Test Sample

Q91 Sample ID

○ Test (1)

Q92 Please rate the degree of red intensity.

	Not intense (turkey frank)	Very intense (all-beef frank)
	0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	
Red intensity ()		

Q93 Please rate the beef flavor intensity.

None Extremely intense

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Beef Flavor ()	

Q94 Please rate the intensity of any seasoning or spices.

None Extremely intense

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Seasoning/Spice ()	

Q95 Please rate the springiness of the sample.

Very soft Very springy

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Springiness ()	

Q96 Please rate the cohesiveness (binding) of the sample.

Not cohesive Extremely cohesive

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Cohesiveness ()	

Q97 Please rate the degree of mouth coating (greasiness) of the sample.

None Excessive

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Mouth coating ()	

Q98 Please rate the intensity of any observable off-odors.

None Extremely intense

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Off-Odor Intensity ()	

Q99 Please describe any off-odors detected.

____

Q100 Please rate the intensity of any off-flavors found in the sample.

	None		Extremely intense
	0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15		
Off-Flavor Intensity ()			

Q101 Please describe any off-flavors detected.

____

End of Block: Test Sample