UTILIZATION OF FROZEN THAWED SEMEN IN LARGE BLACK PIGS; GROWTH AND CARCASS CHARACTERISTICS OF LARGE BLACK PIGS FED DIETS SUPPLEMENTED WITH OR WITHOUT ALFALFA

by

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Dedicated to my Aunt Eunice (1925-2020)

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LIST OF ABBREVIATIONS

ADFI	average daily feed intake
ADG	average daily gain
AI	artificial insemination
ART	assisted reproductive technologies
CAI	conventional artificial insemination
CON	control diet
DIUI	deep intra uterine insemination
DS	Duroc sired
FIB	fiber diet
FTAI	fixed time artificial insemination
FTS	frozen-thawed semen
G:F	gain to feed
HCW	hot carcass weight
IUI	intra uterine insemination
LB	Large Black sired
LC	Livestock Conservancy
LEA	loin eye area
LMF	last Matrix feeding
PCAI	post cervical artificial insemination
SFTAI	single fixed time artificial insemination
USDA NAGP	USDA National Animal Germplasm Program
WBSF	Warner Bratzler Shear Force

ABSTRACT

In recent years conservation of minor livestock breeds has been faced with numerous challenges attributed to decreasing national herd sizes, as well as differences in reproduction and growth. One such minor swine breed, the Large Black pig (LB), is increasingly attractive to small farmers due to their foraging abilities and carcass characteristics. Therefore, the LB pigs have been used in niche pork production systems which market pasture-raised pork products. The LB breed is critically endangered, maintaining a registered breeding population of less than 400 animals, with increasing prevalence of inbreeding and genetic drift. Therefore, the LB breed could benefit from a genetic importation to increase genetic diversity in a national herd with rapidly decreasing animal numbers. A genetic importation would require frozen semen to be brought in from another country for use in breeding U.S. pigs. Frozen-thawed semen (FTS) presents challenges for swine due to the reduced motile sperm cells which negatively impacts fertility. Therefore, the present study evaluated the utilization of FTS in a genetic importation for the LB pig.

A genetic importation occurred in 2016 where semen from the United Kingdom was used on various farms in the U.S. but resulted in zero piglets born. Therefore, 16 LB sows were donated to Purdue University for research into improving estrous and ovulation synchronization to facilitate FTS utilization. Four breeding replicates were performed where following 14 days of Matrix feeding, OvuGel® was administered at 144 h following last Matrix feeding (LMF) or 96 h in post-weaned sows and two FTS inseminations occurring at: 30 and 36 h, 17 and 23 h, 24 and 30 h, and 24 and 32 h after OvuGel® for replicates 1-4, respectively. Approximately 2.64±0.3 billion motile sperm cells per insemination were utilized in replicates 1-3 with American LB FTS, with replicate 4 utilizing 0.34±0.03 billion motile sperm cells of imported FTS. Follicle diameter (P=0.260), ovulation within 48 h of OvuGel® (P=0.411), and weight prior to breeding (P=0.681) did not influence conception rate, however expression of estrus was determined to significantly influence conception rate (P=0.043). Seventy-five LB piglets were weaned across the first three breeding replicates, with parity 2 sows observed to have larger litter sizes than parity 1 sows (P=0.066).

Large Black and Duroc-sired (DS) crossbred pigs from replicates 1 and 2 farrowing were fed corn and soybean meal based finishing diets supplemented with (FIB) or without alfalfa and wheat middlings (CON). Following 6 dietary phases through finishing, 25 LB and 25 DS pigs were

slaughtered at similar ages for digestive organ dissection and carcass measurements. Loin muscles were evaluated for fresh pork quality and instrumental color and tenderness. LB pigs had a reduced ADG (P<0.0001) and G:F (P<0.0001) compared to DS pigs. Pigs fed FIB resulted in reduced ADG (P=0.020) and reduced G:F (P=0.007). At slaughter LB pigs were 26.4 kg lighter than DS pigs (P<0.0001), and pigs that were fed FIB had lighter live weights (P=0.002) than pigs fed CON. LB pigs had 28.5±1.3 cm^2 smaller longissimus muscle area (P<0.0001), yielding 2.0 cm more 10^{th} rib back fat than DS pigs (P<0.0001). CON pigs had heavier HCW (P<0.0001) than FIB pigs, however FIB pigs had greater percent lean (P=0.015). LB pigs had significantly reduced percent lean than DS pigs (P<0.0001). LB pigs had loins with reduced drip loss (P=0.009) and cooked shear force values (P<0.0001). Overall, the growth and carcass composition of the pigs was most affected by genotype, and to a lesser extent than the type of diet fed.

In conclusion, the genetic importation of LB semen was successful as ½ blood piglets were created for dispersal into the U.S. LB herd. Improvements in FTS utilization in this heritage breed contributed to the successful creation of live-born pigs. Additionally, growth and carcass information was obtained for LB breeders to use in understanding and marketing of this heritage breed of pigs.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

In recent years, conservation of livestock breeds has become a priority to prevent the potential loss of genetic material that rare livestock breeds contain. In the United States, there are at least ten minor breeds of swine that have established relatively small breeding populations known as heritage breeds, due to the historical significance of these breeds. In this literature review, heritage breeds will be referred to as minor breeds due to the wide variety of terminology to describe heritage breeds. Some of these breeds are so few in number that they are considered to be an endangered breed. With the adoption of reproductive and genomic technologies, conservation of these minor breeds has the potential to evolve and have increased success. The Large Black pig, one of the minor breeds in the U.S., has been characterized as endangered (threatened) by the non-profit Livestock Conservancy, and has great potential for benefit from modern conservation efforts.

1.2 Description and History of the Large Black in Relation to the U.S. Swine Industry

The Large Black swine breed is an all-black pig recognizable and distinguished by its large lop ears that extend downwards to its nose, with a Large Black sow profile provided in Figure 1.1. The United Kingdom Large Black breed standard is described in 1913 as "whole black, the skin fine and soft, covered with moderate silky hair" and "the head should be of medium length, wide (head) between ears covering the face" (Wallace, 1913). Described in the early 20th century to be "rapid in growth", sows have been reported to reach weights that are 227 to 272 kilograms (kg) (500 to 600 lbs) with boars reaching weights that are 318 to 363 kg (700 to 800 lbs) according to the Livestock Conservancy. Figure 1.2 depicts a photo of a Large Black sow representing the early United Kingdom Large Black breed standard taken at the Peterborough Show, an early 20th century pig show. The Large Black has been described as maintaining a calm temperament, thought to be due to the ears covering its face obstructing its vision (Wallace, 1913) making this breed attractive to homesteading and low input farmers. The ears are thought to protect the eyes from dirt and debris while rooting for food sources. The native Large Black sow appears to have shorter ear lengths and stockier body condition, while the present day American Large Black sow appears to

have longer ear length and thinner body condition. The body condition difference may be attributed to different feeding management practices.

Figure 1.1. Photo of American Large Black sow in 2017. Photo Courtesy of Dr. Kara Stewart, Purdue University, West Lafayette, IN.

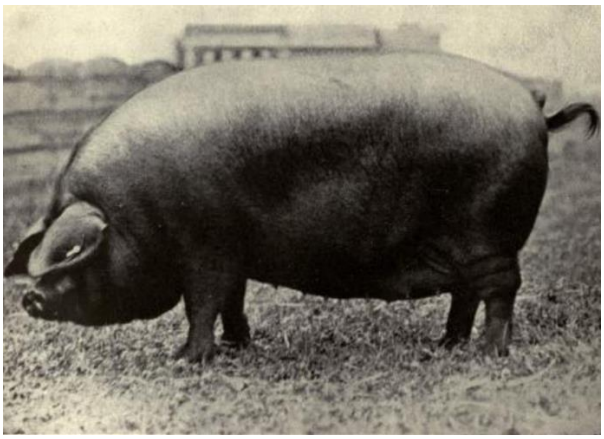

Figure 1.2. Photo of native Large Black sow, "Large Black Sow, 'Hasketon Long Bess', First Prize, Peterborough Show, 1910." Photo from British Breeds of Livestock, 2nd Edition, Board of Agriculture and Fisheries, London, 1913.

The Large Black breed is descended from the now extinct Old English Black pig crossed to various black pigs imported from Asia first described in the early 1800s. Today's Large Black breed has a history dating back to 1898 when a breed association formed in its native Cornwall and Devon in southwestern United Kingdom, where two distinct Large Black populations originated (British Pig Association, 2019; Dohner, 2001). An early British breed handbook notes that the Large Black originated from various populations in Suffolk and Essex counties in addition to Cornwall and Devon counties with highly variable breed traits between each population (Wallace, 1913). The Large Black is also known as the Cornwall Black or Devon Black in other parts of the world. The Large Black was exported out of the United Kingdom to numerous countries in the early 20th century, resulting in the Bo Cake pig breed originating from imported Large Black pigs in 1940 to Burma and also forming the genetic basis for the Canastrao breed of Brazil (Porter et al., 2016). In comparison, the ancestors of today's American commercial swine breeds entered the United States as early as the 1800s (Welsh et al., 2010), with the Large Black breeds first imports being relatively recent beginning in the early 1900s. The first records of the Large Black's importation to the United States as early as the 1920s from Canada, but by the 1960s,

the breed lost interest among American pork producers (Livestock Conservancy, 2020b). This loss of interest has been thought to be due to the Large Black's dark pigmented hair, undesired by processors due to an extended scalding process and most likely due to lack of consumer interest in pig lard (Dohner, 2001; Porter et al., 2016).

By the 1960s and 1970s, the U.S. swine industry underwent rapid production changes from extensive to intensive production systems. During this same time, the U.S. Large Black population was not an established breed for traits such as growth and meat quality, leaving the Large Black breed undesirable in confinement environments (Dohner, 2001; Livestock Conservancy, 2020b). As the American Large Black breeding population continued to decline, the Howitt report was released in 1955 by the British government that discouraged the rearing of the Large Black pig breed and other notable minor pig breeds that are considered endangered today in an attempt to improve the productivity of the British swine industry (Porter et al., 2016; RBST, 2020). In 1985, Ag-World Exports performed a genetic importation in an attempt to introduce Large Black genetics to commercial pork producers to increase hybrid vigor to the U.S. swine herd (OSU, 2015). However, this did little to impact the commercial swine industry due to slow growth rates of the Large Black pigs. In the 1990s, pork prices dramatically declined, leading to further consolidation in the swine industry (Honeyman et al., 2006). By December 1998, the industry witnessed record low hog prices, reaching $15 per hundred weight (cwt) with many pork producers unable to continue production (Fabiosa, 2015). In order to remain in production, some farms changed marketing strategies focusing on raising minor breeds for local or niche markets (Honeyman, 2006).

The Large Black is commonly listed as a minor livestock breed, or a traditional livestock breed that were selected and adapted to local environments retaining unique traits (Livestock Conservancy, 2020a). Heritage breed associations and organizations like the Livestock Conservancy suggest that heritage is an art, and not a science. Heritage is commonly interchanged with other terms such as "historical", "endangered", or "native" to describe these livestock breeds, which have maintained relatively small populations. Other terms that may describe heritage breeds, may be "minor" or "low input" breeds due to their perceived popularity among extensive production systems (Herrero-Medrano et al., 2014). Some minor breeds are thought to contain beneficial traits, such as stress and disease resistance, and unique carcass qualities (Ratky et al., 2013), attributed to each breed's ability to adapt to extreme environments. In recent years,

Berkshire pork has been defined as “heritage” or “heirloom”, however the Berkshire breed is not classified as endangered by the Livestock Conservancy, widely raised in the United States, and exported to Japan due to its pork marbling qualities (Honeyman et al., 2006). Heritage marketing has been disputed by the Livestock Conservancy and minor breed associations that have requested the USDA to recognize a heritage certification, however it has yet to be granted. The National Pork Board (NPB) defines heritage pork production as niche or alternative pork, which is broadly defined as “pork with certain attributes not found in traditional commodity pork” that consumer preferences may be willing to pay a premium for (NPB, 2020). The NPB has yet to define heritage a designated market. In regards to the Large Black, the breed has been recorded to have unique foraging and meat quality traits (OSU, 2015; Livestock Conservancy, 2020b), that have become marketable in niche production, such as in organic, local- raised, and heritage breed specific marketing of pork products.

1.2.1 Carcass

In order for minor breeds to remain productive, carcass qualities are often marketed as being different than that of major swine breeds. Marketing may rely on unique breed traits, management standards, or a combination of both described by Honeyman et al. (2006). Minor breeds are thought to contain different carcass traits such as darker pork color and abundant marbling. Though not a minor breed, Berkshire pigs have been prized for their carcass traits that have been marketed in the U.S., and prized in Asian markets especially Japan (Oh and See, 2012). Like minor breeds, Berkshire loin eye sizes are much smaller than major swine breeds like Duroc (Suzuki et al., 2003). Through heterosis, crossing minor breeds to major breeds, these pork qualities could be utilized in major swine breeds to increase fat depositions in lean pork. Whitley et al. (2012) found that the Yorkshire crossbreds sired by Large Blacks had smaller loin eye areas than purebred Yorkshires. There is a perceived heterosis advantage minor breeds could pose for pork producers, however past research has found that it comes at the expense of lean growth which is not desired by pork producers (Park et al., 2017). There are few pork producers interested in increasing backfat and intramuscular fat, characteristics minor swine breeds present. Currently, there is little to no information on carcass composition and characteristics of purebred Large Black pigs. There is a need to further understand minor breed carcass qualities to assist with conservation efforts.

1.3 Conservation of Minor Breeds

Minor pig breeds today face many challenges including small population sizes, decreased genetic diversity, and support for conservation efforts. In order for conservation to be successful, many different entities must commit to the efforts to save the breed including farmers, government, and non-government agencies. Since there are fewer American farms (USDA NASS, 2019), the number of small farms that raise heritage pigs has continually declined in the U.S.. Fewer and fewer Americans are raised in rural towns which contributes to lack of interest and knowledge about livestock, likely contributing to the decline in heritage pig farms. Some conservation efforts in genetic diversity can consist of sharing genetics between farmers within the U.S. while others require importation of genetics from outside of the U.S.. Regardless of conservation strategy, both of these require money and understanding of methods to increase genetic diversity.

1.3.1 Genetics and Inbreeding

In modern pig breeds, genetic selection has focused primarily on lean growth and litter size characteristics for the last 20 to 30 years. This is true for both maternal genetic lines and terminal genetic lines. However, in maternal lines, selection pressure to produce higher numbers of piglets in each litter has also been included. Specifically, maternal selection pressures were placed on decreasing sow non-productive days and increasing piglets per sow per year. Other traits have also been subjected to selection pressures such as age at puberty or age at first estrus (Schukken et al., 1992). These advances in genetic selection resulted in enhanced reproductive performance where the number of piglets born has increased from 11.3 in 2001 to 13.4 in 2012 (Kraeling and Webel, 2015).

Minor breeds are thought to be resemble pigs before intense genetic selection was employed in the U.S. swine industry and abroad, and often have been described as sources of added hybrid vigor in today's swine industry which continues to be explored by a growing number of pork producers. For example, the Meishan has been prized as prolific, producing very large litter sizes (Knox and Wilson, 2006) with gilts attaining puberty as young as 100 days of age. If crossed with a modern-day pig selected for growth rates and carcass traits, reproductive performance may be improved, however, it would come at the expense of lean growth. Genetic selection in minor breeds has not been focused on lean growth, and genetic selection may be lacking all together.

Defined as the effective population size, Ne, is a quantitative measure of a particular species' genetic variation. A low Ne value results in reduced genetic variation, resulting in an increased risk of inbreeding depression (reviewed by Kristensen et al., 2015) resulting in a loss of fitness. The United Nations Food and Agriculture Organization recommends each population maintain at least 50 individuals per generation, however others have suggested effective population sizes greater than 50 may be more beneficial for minor pig breeds (FAO, 2012). Interestingly, genetic selection in small populations is attainable, however criteria must be highly selective to prevent loss of beneficial traits. Past strategies have relied on breeding programs set to continuously rotate animals between farms, also known as a cyclic breeding system as seen in the endangered Gloucestershire Old Spots pig breed (Livestock Conservancy, 2020c). It is highly recommended to continuously replace boars frequently to reduce inbreeding in minor pig breeds (Christman and Sponenberg, 1995). With the decline in numbers of purebred heritage pigs in the U.S., maintaining enough animals to get an effective population is challenging.

Minor pig breeds that maintain smaller population sizes are at elevated risk of loss of genetic diversity in comparison to commercial swine breeds. Genetic diversity can be measured by the inbreeding coefficient, which utilizes the pedigree of the animal to determine the probability that two alleles at a particular loci are the same, or by testing for homozygosity of alleles. The inbreeding levels of both sire and dam have been studied in the past, and have found that the inbreeding of sires has had little to no effect on litter traits. It was found that the inbreeding of Large White dams significantly decreased birth weights, and consequently weaning weights, while inbred sires had little or no influence on litter characteristics (Bereskin et al., 1968). One study found that it took an additional 3.2 days to reach 104 kg of body weight per 10% of inbreeding in purebred Hampshire pigs, which decreased the performance and potentially the profitability of the purebred operations analyzed (Culbertson et al., 1997). This suggests that intensive maintenance of pedigree records is crucial for overall breed performance and longevity in production.

Increases of inbreeding has been observed in minor swine breeds, increasing the incidence of debilitating genetic mutations. Not all mutations are classified as damaging, however small population numbers pose greater risk for increased frequency of non-beneficial mutations harming overall population fitness. Herrero-Medrano et al. (2014) conducted a study evaluating both commercial and minor breed genome sequences, finding minor breeds with elevated levels of inbreeding. Of the minor breeds evaluated, Mangalitsa was concluded to have limited genetic

diversity with Large Blacks being intermediate, and British Saddlebacks being the most diverse from a Porcine SNP60 BeadChip analysis. Increased repetitions of mutations in genomes can cause a greater occurrence of debilitating genetic factors such as genetic drift and bottleneck. A genetic drift may occur with an adequate population size, when a desired trait may be favored and its frequency leads to loss of another gene leading to increased homozygosity, or inbreeding (FAO, 2012). A genetic bottleneck occurs when there is a loss of individuals decreasing effective population size (Ne) which could result in risk of extinction. The AZGP1 gene was noted to contain a debilitating mutation found in the Mangalitsa, Gloucestershire Old Spots, and European Wild Boar, possibly damaging the AZGP1 gene that is related to number of vertebrae, ear shape and abdominal fat (Herrero-Medrano et al., 2014). Damaging mutations have a greater tendency to be found in genomes with highest levels of inbreeding in Mangalitsa, Tamworth and Gloucestershire Old Spots breeds. Traits associated with growth and carcass, were also found to be overlapping with increased homozygosity mostly in breeds with decreased genetic diversity such as the Tamworth and Gloucestershire Old Spots (Herrero-Medrano et al., 2014).

1.3.2 *In situ* Conservation

In situ conservation programs of genetic resources are costly to manage in their native habitats requiring higher population sizes (Ne), with this being the primary conservation method of animal and plant germplasm material. *In situ* programs are maintained by a wide variety of stakeholders which may include government, non-government organizations, and private individuals. Numerous conservation programs maintain live populations on the basis of genetic potentials such as disease resistance, climate adaptations, and cultural reasons (Henson, 1992). Often times, breed conservation rely on private individuals due to the higher costs of inputs required to raise these breeds. Private individuals have been noted as crucial for initiating conservation efforts for increasingly uneconomical or minor breeds to survive until conservation efforts may begin (Henson, 1992). Individuals and private organizations have been credited as the most successful conservation programs for conservation of minor breeds of species.

There are challenges associated with individuals running conservation efforts. As it is more expensive to manage minor breeds, breeder turnover among minor livestock breeders has been a challenge in conserving minor livestock breeds. Maiwashe et al. (2004) examined the effect of breeder dynamics on breed conservation efforts, specifically in the endangered Navajo Churro

sheep. Navajo Churro sheep maintain a small breed association with a small registered breeder base. After examining the stayability of registered breeders, findings indicate that large numbers of breeders tend to raise Navajo Churro sheep for shorter amounts of time. In the described conservation strategy of the Navajo Churro, long term breeders are the primary conservationists that positively impact the Navajo Churro breed by maintaining animal numbers and preserve herds (Maiwashe and Blackburn, 2004). Minor pig breeds are similar to the Navajo Churro sheep breed, evident in the Large Black population size. The recent Large Black breed census suggests there are less than 50 active breeders (Payne and Couch, 2020), indicating high breeder turnover. Implementation of a genetic management plan relies on long term breeders to maintain the Large Black's limited genetic base.

1.3.3 Role of The Livestock Conservancy

Formerly known as the American Livestock Breed Conservancy, the Livestock Conservancy (Pittsboro, NC) is a nonprofit organization formed in 1977 concerned with conservation of endangered livestock and poultry breeds. The Livestock Conservancy (LC) maintains a conservation priority list of livestock breeds as defined by its specific parameters. The Livestock Conservancy assigns conservation status based on specific criteria (Livestock Conservancy, 2020a) that includes: presence in the U.S. for 100 years or since 1925, contains pedigree documentation, and previous population census findings. LC ranks the census findings based on one of five categories: "Critical", "Threatened", "Watch", "Recovering", and "Study". "Critical" endangerment status requires less than 200 annual registrations, estimating a global population of 2000. "Threatened" requires less than 1000 annual registrations, estimating a global population of 5000. "Watch" status requires less than 2500 annual registrations, and "Recovering" status requirements exceed the "Watch" category. The "Study" category is for breeds that lack all documentation or is endangered outside of the United States. Prior to the establishment of the USDA ARS National Germplasm Program, the Livestock Conservancy maintained a minor gene-bank, but has since released its germplasm to the USDA ARS for germplasm banking (CAST, 2019). In recent years, the Livestock Conservancy has focused on the development of endangered minor livestock breed marketing ensuring breed continuation through marketing of productive meat and wool products, and engaging future livestock breeders.

1.3.4 *Ex situ* Conservation

Ex situ conservation is defined as preservation of genetic resources in storage by method of cryopreservation in liquid nitrogen stored at or below -196 °C. Reasons for cryopreservation of valuable genetic tissues are to protect genetic resources from breeding mistakes, disease concerns, and natural disasters. Reasons for conservation of a livestock species may include, unique traits, historical or cultural context, or economically important traits (FAO, 2007). This ensures populations maintain a protected source of genetics that can be readily utilized when needed by both private and public sector. In relation to inbreeding concerns, cryopreservation of semen and ova is considered one of the best methods to reduce debilitating incidences of inbreeding, effective by increasing generation interval between grand-parent and progeny (Kristensen et al, 2015). Cryopreservation of semen is the most effective method to increase the generation interval between individuals. Cryopreservation of boar semen has been proven as the most practical long-term storage method of semen. Potential benefits of cryopreservation include genetic preservation but are not limited to, herd health protection, elite genetic selection, and long-distance transportation of genetic material (Bailey et al, 2008; Yeste, 2015).

1.3.5 The Case of the Mangalitsa

In some unique cases of herd preservation, it has been recorded that traditional reproductive management practices used in commercial swine breeds, may not be completely transferable to minor swine breeds. In 1975, it was observed that there were only 34 registered Mangalitsa sows located in its native Hungary. The Mangalitsa is commonly cited as a fat pig breed popular among low input farming (Ratky et al., 2013). Following the post-World War II era, consumer demand switched from lard type to lean breeds of pigs, the Mangalitsa almost became extinct until conservation efforts were started by government officials, universities, and private individuals who funded herd preservation research projects amid a renewed interest in the breed as a premium pork product in the 1990s. In a review of reproductive physiology characteristics and application of reproductive technologies in Mangalitsa pigs, it was reported that Mangalitsa females ovulate fewer oocytes without hormone treatment intervention, and observed fewer gilts showing cyclicity at slaughter reported between the ages of 12 to 15 months, which could potentially interfere with cryopreservation and herd preservation actions (Ratky et al., 2013; Egerszegi et al., 2003).

Additionally, it was found that though uterine horn length and weights were similar to Landrace gilts, the weight of the Mangalitsa uterus increases in size much later in gestation (days 12 to 24) compared to Landrace gilts (days 1 to 12), with the authors suggesting that this could be a direct cause of Mangalitsa litter sizes being reported around 7 pigs per litter (Ratky et al., 2013). It has been reported that in one study that only 40% of boars were able to be successfully trained, which could potentially hamper conservation and cryopreservation attempts (reviewed by Ratky et al., 2013). Evident in Mangalitsa breed research, minor breeds may contrast sharply to major commercial breeds among reproductive performance traits attributed to differences in individual behavior, physiology, and management systems. Therefore, reproductive management of minor breeds, like the Mangalitsa, may require a more thorough understanding of unique breed characteristics, potentially benefiting herd preservation programs.

1.3.6 Role of the USDA National Animal Germplasm Program

Developed countries, specifically the United States, are at risk of losing diverse genetic material found in rare livestock species despite containing the largest livestock sector (CAST, 2019) because livestock industries have relied mainly on only a few major breeds. However, developing countries are at even higher risks of losing native livestock species due to genetic importations from developed countries as attempts to introduce proven genetics in the developing countries livestock industries (FAO, 2007; Blackburn, 2009a). In response to conservation of genetic resource concerns, legislation in 1990 was passed to create the animal germplasm division at the National Center for Genetic Resources Preservation located in Fort Collins, Colorado. By 1999, the USDA ARS National Animal Germplasm Program (NAGP) was formed to conserve animal genetic resources as the United States primary animal gene bank (Blackburn, 2009a). The USDA NAGP has since collected primarily germplasm and tissue, that provides both the private and public sectors genetic material storage options.

1.3.7 Past Genetic Importations

Recently as concerns over animal genetic diversity have come into question, several minor livestock breeds such as the Large Black have become increasingly in danger of extinction. Minor livestock breeds maintain relatively low breeding population numbers, yet high interest among

low input farmers. Faria et al. (2019) analyzed the genetic diversity of several pig breeds in the United States and found that minor pig breeds share similar average inbreeding levels compared to major swine breeds. Inbreeding and small population numbers are challenges unique only to minor pig breeds. In order to assist in the flow of new genes into a population or to establish an entirely new population, genetic importations have been attempted.

In the 1980s, the Meishan pig was imported into the United States from its native China to provide beneficial reproductive performance traits, however the Meishan pig was not widely accepted due to the Meishan pork quality differences observed in previous studies of growth and composition (White et al., 1995). In a case study analysis of two distinct randomly bred US research populations, the impact of genetic drift on the Meishan populations was determined to be a major factor. Genetic drift is the tendency for a small population of animals to experience fewer diverse traits leading to a genetic bottleneck. It was determined that the native Chinese Meishan had higher levels of diversity than the U.S. populations, indicating a higher level of inbreeding has occurred in the U.S. population (Blackburn et al., 2014). Genetic drift is an important consideration when managing livestock. It is best to employ a degree of genetic selection while decreasing inbreeding to avoid genetic drift, or valuable traits will be lost to unmanaged pedigrees.

There have been several past genetic importations with varying degrees of success and failures. One study analyzed pedigree records to trace the number of registered progeny of imported animals from both new breeds and new animals within an established U.S. breed (Blackburn and Gollin, 2009b). Jersey and Limosin cattle were imported to the U.S. to provide new genetics for their established populations. The Limosin cattle imports became infrequent, and resulted in a decline in number of registered progeny from imported cattle, with a conclusion that it became undesired by breeders to import new genetics but rather to continue improving the cattle that were already present. In regards to Jersey cattle, after the initial imports from France, the number of descendants of the imported Jersey cattle decreased after three generations. The U.S. bulls outperformed the imported bulls in milk production, even with the imported bulls having higher production yields (Blackburn and Gollin, 2009b). As an example of a new breed introduced in the U.S., both Boer goats and Meishan pigs were imported for their unique traits, however the Boer goat is the only new breed import that has been relatively successful with the Meishan considered a failed import discussed earlier. The Boer goat has been determined successful with a competitive number of registered progeny becoming a popular goat breed, which have desirable

rapid growth rates. Likewise, reason for the Boer goat being a success when compared to the Meishan is that it contains desirable breed characteristics that the Meishan does not have. Though the single reproductive trait that Meishan have is highly valued, the associated negative consequences of carcass traits are a trade-off that pork producers are unwilling to invest in. Positive traits that the Boer goats contain traits such as rapid growth rates that are of economic value and enable the breed to distinguish itself, and become desired by producers. If an animal is introduced through an importation, then that individual must be able to compete with multiple positive traits to be able to make an impact on the U.S. population (Blackburn and Gollin, 2009b). In conclusion, descendants of imported progeny may not compete among native animals with evolving breeder interests and consumer demands.

In order for the Large Black importation to be successful, several factors need to be considered and managed: (1) successful creation and dispersal of imported genetic offspring in the U.S., (2) efforts must be cooperative in the management of both American and imported Large Black lines, and (3) implementation of a degree of genetic selection for economically and distinguishable traits in the overall Large Black population to reduce the risks for genetic bottleneck and drift. It is not known how the imported Large Black will perform among the number of breeders that are invested in the import. There are several concerns that the imported Large Black genetics could pose a threat to American genetic lines, which may only be prevented by an adequate breed management plan for the breeders to follow to properly manage the imported genetics along with the American Large Black population.

1.3.8 Recent History of the Large Black

In 1998, Cabbage Hill Farm (Mount Kisco, New York) facilitated a live animal import from the United Kingdom, becoming the first pedigrees recorded by the North American Large Black Pig Society. The North American Large Black Pig Society was formed by Ted Smith, Still Meadows Farm of Laurel, Mississippi, in 1999 registering Large Black populations in both the United States and Canada until a breed association was chartered in 2009. In 2008, a Rare Breed Swine Initiative Meeting was held in Columbia, Missouri beginning a series of breed conservation discussions and actions concerning the threatened endangerment status the Large Black breed maintains. The Large Black Hog Association (LBHA) formed in 2009 first officially using the term “hog” in its name, incorporating pigs from the North American Large Black Pig Society

registry (LBHA, 2020). In 2016, the International Large Black Pig Registry (ILBPR) was formed by Lucky George Farm located in Derby, Iowa. The ILBPR was formed as a separate herd-book to incorporate and register British Pig Association pedigrees bred in the United States from pigs imported by Lucky George Farm. Today, Large Black pigs are found in several countries besides the United States, with all purebred populations endangered evident in breed registry censuses located in Australia, and in its native United Kingdom. Numerous American Large Blacks have been exported to several countries such as South Korea, China, and Philippines.

The Large Black is considered one of the rarest pig breeds with both the United States and United Kingdom reporting small registered populations fewer than 400 registered breeding animals (BPA, 2020; LBHA, 2020). Annual American Large Black registrations have varied in recent years between threatened to critical status on the Livestock Conservancy's conservation priority list (Bryan, 2014). A recent breed association survey conducted by the Livestock Conservancy in 2019 indicates that the Large Black may potentially be critically endangered cited to be due to limited registered breeding population and breeder turnover leaving only a total of 323 boars and sows registered, of which only 25 were new registered pigs (Payne and Couch, 2020). Conservation efforts have primarily focused on registered livestock with pedigree information, and findings have concluded the Large Black population has maintained an elevated inbreeding coefficient of 11.94% from 2009 to 2014 (Bryan, 2014). An inbreeding coefficient is a measure of the degree of homozygosity a group of individuals share (Porter et al., 2016), and has been of concern among breeders of minor breeds such as the Large Black. In response to the extremely low numbers of animals as well as the inbreeding levels of the animals in the U.S., a group of American Large Black and Gloucestershire Old Spots breeders formed the collective: Assisted Reproductive Technologies in Heritage Swine (Heritage Swine Initiative). The purpose of this cooperative effort was to facilitate an importation of frozen boar semen from Deerpark Pedigree Pigs located in the United Kingdom in 2015. Private donors funded the importation process and future germplasm storage at the USDA National Germplasm Program (USDA NAGP).

The USDA NAGP and the Livestock Conservancy partnered and developed guidelines for imported semen dispersal and use among Large Black and Gloucestershire Old Spots breeders in 2016. This plan provided University and industry AI expertise conducting on-farm insemination, formed by contracts with individual breeders in return to provide piglet data tracking the United Kingdom-sired offspring to the USDA NAGP. Between January 2017 and February 2018, 12

Large Black females at 5 locations across the U.S. were inseminated with the imported semen by experts from Purdue University, University of Missouri (MU), University of California-Davis (UCD), University of Pennsylvania (UP) and International Boar Semen (IBS) with results described in Table 1.1. All breeding attempts in 2017 resulted in 0 live piglets born from both Large Black and Gloucestershire Old Spots breeding attempts utilizing the imported semen. Therefore, additional research was needed to identify methods to successfully create piglets from the imported frozen semen.

Table 1.1. 2017 Imported semen breeding attempts

Sow Origin[1]	Number of Sows	UK Boar Line	Motility (%)	Number of Conceptions
Ohio	1	Super	30	0
Iowa	8	Super & Malcolm	-	5
Oregon	2	Super & Malcolm	15-20	0
Pennsylvania	1	Malcolm	-	0
Kansas[2]	1	-	-	0

[1]Information from H. Blackburn (USDA NAGP, Fort Collins, CO, personal communication); W.L. Singleton (Purdue University, West Lafayette, IN, personal communication).

[2]Gloucestershire Old Spots mating only.

1.4 Assisted Reproductive Technologies and Frozen Thawed Semen (FTS)

In order to produce enough pork to meet growing consumer demand for animal protein products, assisted reproductive technologies have been implemented to increase reproductive efficiency which has resulted in the ability to produce and market around 115 million pigs per year in the United States (NPPC, 2020). Pork is consistently one of the most consumed animal protein products in the world (FAO, 2020), with demand continuing to remain constant. Therefore, it is pertinent to produce enough pigs each year to meet worldwide demand as the world's population continues to grow. Around 26% of the pork produced in the United States is exported to other countries (NPPC, 2020), due to the reduced cost of production for U.S. pork producers in comparison to other countries. Assisted reproductive technologies have impacted the U.S. swine industry, with more than 95% of all U.S. producers (Knox, 2016) utilizing artificial insemination (AI) today to meet pork product demand. As the U.S. swine industry looks to improve efficiency through progressive management practices, there is always a growing need for sustainability. Like other animal agriculture industries, today's U.S. swine industry is able to produce more pork with

less animals through a combination of progressive management tools, including assisted reproductive technologies (ART). Cryopreservation of boar semen has great potential to impact the U.S. swine industry. Currently, the efficiency of FTS has not matched that of fresh semen, therefore, its adoption into commercial farms is limited.

1.4.1 Utilization of Frozen Boar Semen

Cryopreservation of boar semen has been proven as the most practical long-term storage method of semen. Potential benefits of cryopreservation include genetic preservation but are not limited to, herd health protection, elite genetic selection, and long-distance transportation of genetic material (Bailey et al., 2008; Yeste, 2015). Cryopreservation presents an excellent method of decreasing inbreeding, and protection safeguard from potential disaster such as disease by providing an efficient method to increase generation intervals between animals. However, due to damage on the sperm cell from cryopreservation and thawing processes, it has not been adopted in commercial production, but maintains as a proven method of genetic preservation for both sperm and embryos.

Despite the long-term storage benefit, it has been estimated that FTS accounts for less than 1% of all artificial inseminations conducted in the United States swine industry (Johnson et al., 2000). Sperm cells are highly vulnerable to numerous factors that may inhibit cell function: most commonly temperature changes and chemical exposure. Boar sperm cells are highly susceptible to temperature changes, especially cold shock, observed in both liquid and frozen storage states (Yeste, 2017; McNamara and Knox, 2013). Due to the extreme temperature changes during freezing and thawing processes, frozen thawed semen results in greatly reduced fertilization capacity, conception rates, and litter sizes leading to lack of utilization in the industry (Johnson et al., 2000). Historically, human sperm cells were discovered to be frozen successfully in 1949, only after the discovery of glycerol's cryoprotectant effect on sperm cells (Polge, 1949). Prior to the 1970s frozen boar semen produced poor conception rates without surgery, but it was not until 1975, that frozen boar semen became commercially available following Pursel and Johnson's modifications to the thawing procedure (Pursel and Johnson, 1975) which improved the fertility of FTS. Rapid cooling induces intracellular and ion releases from the sperm cell membrane compromising the membrane integrity. Boar sperm cells contain lower cholesterol to phospholipid ratios, compared to the cryotolerant bull sperm cell (Parks et al., 1992) and when combined with

rapid temperature shock, subjects the boar sperm cell to membrane damage (Watson, 2000; Flores et al., 2008) with ice crystal formation. Flores et al. (2008) concluded that the damage sustained during the cooling process alters mitochondria function, impairing sperm cell function, and subsequent fertility. Cellular mitochondria function is crucial for proper cell function, producing energy for processes like cellular respiration.

While boar sperm cells are less tolerant to the freezing and thawing process than other species, there is additional evidence that boar semen fertility using FTS varies among individual boars. Salamons et al. (1973) studied the effects of centrifugation, diluent and pellet volume on post-thaw motility and found that the Berkshire boar had higher post thaw motility compared to the Large White. The Large White was found to perform better with an egg yolk diluent, however this study used a small sample size of only of 3 boars. Additional studies have accounted for individual boar variation (Waterhouse et al., 2006), with indication that there is post-thaw motility variation between ejaculates of the same boar (Knox and Yantis, 2014, Spencer et al., 2010).

Additional differences in individual boars comes from the presence of specific proteins associated with the sperm plasma membrane which have been associated with cryotolerance. Llavadra et al., (2019) identified several transmembrane proteins on the sperm cells that were associated with cryotolerance (Llavadra et al., 2019) in the individual boar rather than in specific breeds. Llavadra et al. (2019) reported findings that concluded that levels of GTSM3 protein may indicate poor binding of sperm to the oocyte during the fertilization process, resulting in reduced conception rates. The relative abundances of IZUMO1 protein were higher in sperm cells prior to freezing, than in sperm post-thawing, but was not a significant indicator of cryotolerance. It's concluded that both GTSM3 and IZUMO1 proteins are displaced due to damage during the cryopreservation process resulting in cellular damage. Cellular damage induced on the surface of the cell is thought to decrease the sperm cell's ability to attach to the ovulated oocyte and in combination with a limited lifespan due to damage and inability to function, limiting fertilization.

1.4.2 Number of Sperm Cells

Due to damage of the sperm cells during freezing and thawing, higher total numbers of sperm cells are required during artificial insemination using FTS compared to liquid semen. FTS doses require between 4 to 5 billion total cells (Bertani et al., 1997; McNamara and Knox, 2013) in comparison to 2 to 3 billion total cells that is required for liquid semen (Didion et al., 2013;

Waberski et al., 1994). However, since the inception of FTS the goal has been to decrease this substantially higher sperm cell requirement in order to improve efficiency. Following Johnson and Pursel (1975) report of utilizing 6 billion cells to achieve conception rates similar to those from liquid semen, there has been a movement in FTS research to decrease the sperm numbers required. The main reason for this is due to high percentage of cells that are essentially wasted during the freezing and thawing processes, that could rather result in more sows inseminated. When evaluating research using FTS, it can be hard to compare studies if the researchers only report total number of sperm cells inseminated because the freezing and thawing processes damages the cells, reducing total motility. It is more useful to compare fertility from total motile cells when evaluating FTS. Liquid semen doses typically average between 2 to 3 billion total cells with average motilities above 75% for conventional AI methods where semen is deposited in the cervix during insemination (Watson, 2000; Didion et al., 2013). More recent research on FTS indicated that single doses of 4 billion cells (McNamara and Knox, 2013) rather than 6 billion cells per two inseminations (Pursel and Johnson, 1975) can produce competitive pregnancy rates to liquid state semen. In this study the post-thaw motilities were reported as 51±3.2 and 42±3.5%. Therefore, about 2.5 to 3.1 billion motile cells would have been used per insemination, resulting in a total of 5.0 to 6.2 billion motile cells.

One study analyzed the effect of number of motile sperm cells and found that two inseminations with 2×10^9 motile sperm cells per insemination was the most efficient use of sperm numbers with a conception rate of 79.2% and 12.6 piglets (Spencer et al., 2010). The same study evaluated two inseminations of 1×10^9 motile sperm cells and found an acceptable conception rate of 70.4% but a reduction in litter size of 11.3 pigs producing the most piglets with limited sperm numbers. McNamara et al. (2013) utilized 4 billion total sperm in doses with good, moderate, and poor post thaw motility classifications making the number of motile sperm inseminated actually $1.7 \times 10^9 \pm 0.1$, $1.2 \times 10^9 \pm 0.2$ and $0.8 \times 10^9 \pm 0.2$ cells per insemination. They found that there was no effect of the post-thaw motility classification on pregnancy rate or number of fetuses. These results suggest that acceptable fertility can be achieved with 4 billion cells regardless of post-thaw quality. Currently, four billion total cells is considered substantially more cells than what is required for liquid semen.

When using liquid semen, the concentration of sperm can be reduced further when semen is placed in the uterus during insemination like in the method of post-cervical AI (PCAI). Watson

and Behan (2002) concluded that 1 x 10^9 cells deposited in the uterus can establish satisfactory pregnancy rates compared to conventional AI (cervical deposition). It can be hypothesized then that when using FTS, uterine deposition may increase conception with the reduced concentrations of viable sperm cells.

Another method to improve fertility with FTS is the utilization of hormone treatments thought to improve uterine contractions to increase the number of sperm cells populating the oviductal reservoir prior to fertilization. Willenburg et al. (2003) compared adding oxytocin or prostaglandin to liquid semen doses and found both improved litter sizes. Knox and Yantis (2014) repeated this with FTS by supplementing $PGF_2\alpha$, and found no benefit to litter size or pregnancy rate. However, in this study, the number of motile sperm cells (0.5, 1 and 2 billion in two inseminations at 24 and 36 hours after estrus) did have an effect on litter size and pregnancy rate. Hormonal treatments to improve fertility with FTS warrants additional research. Current knowledge would suggest that fertility is compromised with FTS with inseminations of less than 2 billion motile sperm cells.

It is thought that fertility using FTS may be impacted by the immune response induced by the female's reproductive tract in direct response to the increase in dead and damaged sperm cells that are placed in the reproductive tract during insemination. When the uterus detects the presence of unknown foreign bodies, such as sperm cells, it stimulates an immune response and phagocytotic activity. Not only are there more dead and defective sperm in the insemination dose of FTS, but the lifespan of FTS in the female's reproductive tract is shorter than that of sperm cells in liquid state semen (Waberski et al., 1994), increasing the numbers of dead sperm that could illicit immune response from the female. It is hypothesized that one role of seminal plasma is to mitigate the female's neutrophil response (Johnson et al., 2000; Yamaguchi et al., 2013), in relation to FTS procedures, seminal plasma is removed prior to freezing and rarely added to FTS doses after thawing. The addition of caffeine has been thought to increase the number of sperm surviving in the uterus by reducing phagocyte activity (Yamaguchi et al., 2013). When the body detects a foreign body, typically the first cells to the site of infiltration are neutrophils which induce inflammation and immediately perform chemotaxis. Chemotaxis occurs when neutrophils overwhelm and surround the infected or intruding cell and consuming it via phagocytosis. FTS sperm cells are highly susceptible to chemotaxis, which in Yamaguchi et al. (2013), it was

determined that the caffeine additive improved the motility and reduced the number of phagocytotic cells in the uterus following testing.

1.4.3 Current Freezing and Thawing Processes

Frozen boar semen has been commercially available to pork producers since 1975, when boar thawing solution or Beltsville thawing solution (BTS) was developed resulting in improved conception rates comparable to liquid semen inseminations. Pursel and Johnson (1975) procedures resulted in 85% pregnancy rates in both treatment groups of heterospermic and homospermic inseminations. BTS provided a better alternative for seminal plasma, which lead to the commercial availability of frozen semen to be utilized. Prior to the wide spread use of straws, pellets were utilized, however, today straws are the preferred packaging method. While Pursel and Johnson were able to produce acceptable conception rates with almost 12 billion total cells split in two inseminations, this was still considered inefficient in comparison to liquid state semen inseminations (Pursel and Johnson, 1975; Johnson et al., 2000). Freezing methods and media have continued to evolve, including the utilization of controlled rate freezing, improving the fertility with FTS.

Currently there are many variations in freezing protocols being used for boar semen. We will discuss the methods from both the USDA NAGP and Dr. Rob Knox's lab at the University of Illinois. When freezing semen, typically collection of only the sperm rich fraction of the ejaculate is collected and not the post-sperm fraction where the majority of the accessory sex gland secretions occur. The ejaculate is then diluted 1:1 in extender and allowed to slow cool to room temperature. The ejaculate is then slowly brought to 17 °C and maintained at that temperature overnight. This allows for shipping of semen from the site of collection to the lab to freeze, if necessary. The next day, the ejaculate is then centrifuged at 800 x g for 12 minutes and the supernatant of semen extender removed. The pelleted sperm cells are then resuspended in a cooling media and slowly cooled to 5 °C for 3 h. The cooled semen is then packaged into straws varying in size from 5 mL to 0.25 mL. After packaging, the straws are placed in a programmable, controlled-rate freezer to bring their temperature down to 2 °C before submerging in liquid nitrogen storage at -196 °C or below.

Thawing an individual straw for use in artificial insemination has been performed many ways with variations in waterbath temperatures and lengths of time the straws are submerged in

the water. In general, a single straw is submerged in a water bath ranging from 42-80 °C for either very short (e.g., 7 seconds) amounts of time or a range between 20-50 seconds. Different size straws and packaging lead to differing thawing procedures and rates. These variations in procedures can make comparing fertility rates from various scientific studies challenging. Yang et al. (2009) has highlighted the importance of standardization of cryopreservation practices in biomedical fish models. However, in boars, no standardized methods have been developed.

1.5 Artificial Insemination and U.S. Swine Industry

Improving fertility with FTS is an area of research with great importance. In order to improve FTS fertility, one must first understand artificial insemination and estrous synchronization. The U.S. swine industry has capitalized on the benefits of artificial insemination to produce cost effective pork products. The adoption of artificial insemination grew in the U.S. in the 1990s to today's estimates of over 95% of matings are derived from artificial insemination (Singleton, 2001; Knox, 2016). Artificial insemination is the most efficient breeding method that allows for rapid gene transfer and genetic change (Knox, 2016), which has been credited for the rapid genetic improvement in the swine industry. The first attempts at artificial insemination were performed by the Italian scientist Spanllanzani, who successfully inseminated a dog with cooled semen in the 1700s (reviewed by Ugur, 2019). Later attempts by researchers resulted in practical uses for livestock, but it was not until the 20th century that AI was incorporated into livestock production practices.

Artificial insemination has resulted in several advantages to the swine industry and other livestock industries. These advantages include but are not limited to improvements in (1) genetics, (2) disease control, (3) animal handling, (4) labor, and (5) reproductive management (Crabo and Dial, 1992; Knox, 2016). Genetic progress in the last few decades has been attributed to the utilization of artificial insemination where higher performing boars have led to rapid genetic change. An individual boar may produce up to 300 mL per ejaculate, however that is dependent on age, breed, and health status. Through the utilization of AI, one ejaculate may be split into enough doses for approximately 20 inseminations, dependent on the concentration and storage method of the semen dose (i.e. frozen vs liquid). Therefore, AI has been successful in improving individual boar utilization in comparison to natural mating. While AI has been adopted for breeding, genetic and genomic tools to identify superior boars have also evolved. This allows for

identification of boars for use in AI programs to enhance genetic progress. Improved genetic selection of sires has led to larger litter sizes, resulting in more pigs per sow per year (Knox, 2016). However, Welsh et al. (2010) cautions that AI may potentially propagate inbreeding in the commercial swine industry. Analyzing five commercial swine breed pedigrees, the number of breed registries peaked in 1990 and subsequently declined. This has been attributed to a decreased industry reliance on individual purebred breeders, and increased reliance on swine genetic companies (Welsh et al., 2010). The analysis found that all pigs representing all 5 commercial breeds analyzed were 10% or less inbred, with all inbreeding levels being assumed to be underestimated.

Artificial insemination has also contributed to improved herd health status, by decreasing the spread of diseases that may be detrimental to all aspects of pork production. Disease may spread through several modes of transmission, however AI decreases the need for introducing new boars into a sow herd, decreasing disease risks. Boar semen may contribute to the spread of diseases like PRRS virus (Porcine Reproductive and Respiratory Syndrome), with boar studs routinely testing for common diseases to prevent further transmission of disease (Crabo and Dial, 1992).

AI is a useful tool for improved animal handling management. Prior to AI, pork producers used natural service mating systems where sows determined to be in estrus were set in pens with boars to be bred. This process was termed hand-mating where a technician helped to guide the boar's penis into the sow's vagina during natural mating in a pen. Although the commercial U.S. swine industry no longer depends on natural service systems, there was an associated risk of animal and personnel injury in these practices. AI has subsequently decreased the number of boars housed on sow farms where currently only enough boars needed for heat detection are housed on sow farms, decreasing the personal injury risk from boars.

Artificial insemination has also impacted the labor force in the swine industry. According to a study by Crabo and Dial (1992), AI relies on trained technicians to accurately detect females in estrus, handle boars, collect semen, and properly inseminate females. In natural service systems, most of these roles are carried out by the boar. Therefore, with artificial insemination a more trained and specialized labor force is required.

AI allows farms to control the timing of conception to better manage large groups of sows. Most farms manage weekly or monthly groups of sows via synchronized weaning events and

subsequent artificial insemination. Because the process of AI is much faster than that of natural or pen mating, more sows can be bred in a single day using AI. This provides a great benefit to the producer to maximize reproductive management. AI comes with some challenges as well such as increased management intensity and organization, and increased need for technical training. Crabo and Dial (1992) suggest that reasons for failed AI implementation are primarily due to improper estrus detection, a common technician error.

Advantages and challenges associated with AI may be different based on the size of the farm. Ichikawa and Koketsu (2011) compared a combination of natural and AI mating and observed increased farrowing rates when used together on a small farm. Am-in et al. (2010) evaluated natural and AI matings in small farm settings with small sow herd sizes consisting of 10 or less sows per farm. Results found that AI improved non return rates to estrus, farrowing rates, and litter sizes in comparison to sows naturally serviced. This study additionally found no impact on distance of semen transport for AI among small farms in Thailand, concluding that small farm herd sizes may be able to provide AI service in limited boar availability situations improving reproductive performance of small sow herds (Am-in et al., 2010). AI has been observed to be beneficial and possible to implement in small herd sizes improving farrowing rates and litter sizes regardless of herd size.

1.5.1 Intrauterine Insemination

Recently, the adoption of intrauterine insemination (IUI), also referred to as post-cervical insemination (PCAI), has been growing in the U.S. swine industry. IUI differs from conventional artificial insemination (CAI) by location of deposition of the semen dose. IUI includes the passage of an inner catheter through the outer, conventional catheter, into the uterine body. In theory, benefits of IUI over CAI would include utilization of lower sperm doses, increasing number of females inseminated by one boar and a decrease in the amount of time required to inseminate a sow (Garcia-Vazquez, 2019). Disadvantages of IUI are associated with additional training requirements for technicians to successfully thread the inner catheter, which can be more challenging in gilts and lower parity sows. Research into how low the concentration of sperm in the IUI insemination dose can go has been equivocal. One study found that under 1 billion per 25 mL dose reduced the number of embryos (Mezalira et al., 2005). Numerous studies have been

conducted to try and elucidate how low the concentration of sperm can go before fertility is impaired using IUI.

Though it has been accepted that AI in pigs requires 2 to 3 billion sperm cells to achieve favorable conception rates greater than 90%, it has been demonstrated in previous research that doses that consist of less than conventional sperm counts using IUI can achieve similar results to CAI sperm doses (Watson and Behan, 2002; Hernandez-Caravaca et al., 2012). In a comparison of IUI and CAI, IUIs achieved higher fertility results than CAI with a low dose of 1 billion sperm cells indicating that post cervical inseminations improve farrowing rate (Watson and Behan, 2002). Several factors may influence IUI success at the farm level. Rozeboom et al. (2004) found no effects of presence of blood or failure to pass the catheter on conception rate, however low doses of 0.5 billion cells resulted in lower farrowing rates. Mezalira et al. (2005) studied the effect of sperm number and backflow when sows were inseminated within 24 hours of ovulation, and determined there is no difference in pregnancy rate or backflow volume among IUI treatments of 0.25, 0.5, and 1 billion cells. Mezalira et al. (2005) suggests that inseminating 0.5 billion cells within 24 hours prior to ovulation is attainable, allowing IUI to improve the fertility of limited sperm doses otherwise detrimental in conventional inseminations.

It is thought that IUI could improve fertility when used with FTS. FTS has a reduced lifespan and fertilizing ability, therefore in theory, placement closer to the site of fertilization as with IUI could improve fertility outcomes. One study compared fertility from IUI and deep intrauterine insemination (DIUI), where sperm are deposited deep into one uterine horn by the utero-tubular junction, using FTS (Buranaamnuay et al., 2011). This study found a satisfactory pregnancy rate of 88% with FTS in both IUI and deep intrauterine insemination (DIUI). However, IUI had higher numbers of embryos present 45 hours after ovulation compared to DIUI (66% vs 31%, respectively). Though this study was comprised of a small sample size, and is confounded by differing sperm dose numbers (2×10^9 IUI and 1×10^9 DIUI), it could be indicative of IUI's ability to improve the efficiency of frozen thawed semen. The potential for IUI or DIUI to improve fertility with FTS warrants additional investigations.

Regardless of insemination method, the number of inseminations per animal has economic implications. Lamberson and Safranski (2000) compared insemination programs using an economic model comparing number of inseminations, hours relative to onset of estrus, and frequency of estrus detection; it was found that protocols with single inseminations resulted in

poorer predicted performance in comparison to two or more inseminations. Four inseminations conducted at 0, 12, 24 and 36 hours yielded the highest farrowing rate and pigs born per sow with frequency of estrus detection not influencing performance, however, this utilized more semen per female which was considered inefficient. Timing of insemination modeled for predicted economic returns is evident that relative to insemination, estrus detection influences farrowing rate and litter size (Lamberson and Safranski, 2000). Traditional artificial insemination is often practiced with one insemination every day that an animal is observed to be in estrus, resulting in 2 to 3 inseminations. Therefore, with the low viability FTS, there is a need to reduce the number of sperm cells and inseminations required to establish pregnancy. Some research has started to look at single inseminations compared to 2 or 3.

Recent research on fixed time insemination utilizing FTS was reported by Spencer et al (2010) and found that number of inseminations had no effect on conception rate, however it influenced the number of fetuses at 28 to 34 days of pregnancy with two inseminations of FTS improving litter size compared to one insemination. Current advances in FTS utilization has evolved into two inseminations targeting ovulation timing, often at a fixed time relative to estrus or ovulation induction. McNamara and Knox (2013) used heterospermic inseminations among different insemination-to-ovulation treatment times. A trend was reported that the timing of insemination relative to ovulation impacted the proportion of fetuses sired by an individual boar (as determined by genotyping). Interestingly, regardless of post-thaw motility when greater than 26%, most of the piglets that were sired came from the first insemination rather than the insemination closest to ovulation. Single inseminations with FTS have yet to be proven widely effective and warrant further investigations. Currently, two inseminations are recommended to improve FTS conception and litter sizes, however the timing of the inseminations has varied between numerous studies. In Ringwelski et al. (2013) it was recommended at the interval of FTS inseminations be an interval of 8 to 16 hours, however this is highly dependent on when ovulation occurs relative to insemination.

All of these modifications to the insemination procedures are designed to place viable semen into the female's reproductive tract prior to ovulation. Knowing when ovulation occurs in relation to the onset of estrus can allow for insemination to occur at the optimal time relative to ovulation and improve fertility. Almeida et al. (2000) estimated that ovulation occurs approximately 43.9±6.23 hours after the onset of estrus. Additionally, they concluded that two

inseminations 24 hours and 36 hours after onset of estrus could produce the best fertility results using FTS. Waberski et al. (1994) determined that the optimal insemination timing relative from estrus to ovulation was 12 hours before ovulation, with 0-4 and 4-8 ranges after ovulation reducing conception results. FTS resulted in favorable conception rates when insemination occurred 8 hours prior to ovulation through 4 hours after ovulation, making the window of time for insemination relative to ovulation smaller for FTS semen compared to cooled semen. Utilizing FTS, Waberski et al. (1994) determined the lifespan of FTS to be 8 hours, and determined that inseminating prior to ovulation provided the highest pregnancy rate compared to at or after ovulation. McNamara et al. (2013) performed AI at 24 and 36 hours after onset of estrus, outside of the 12 hour insemination to ovulation interval, and found conception rates to reach 75.6±6.8% using moderate to good post-thaw motility, ranging from 26% to ≥40%. This study found the majority of the piglets sired with the first insemination at 24 hours after onset of estrus.

1.5.2 Control of Estrus and Ovulation

Control of the estrous cycle through estrous synchronization is important for the success of artificial insemination because the timing of the insemination relies heavily on the identification of estrus in the female as a predictor of ovulation timing. AI is most successful when sperm are in the females' reproductive tract 12 hours before ovulation (Waberski et al., 1994). Because FTS has a reduced lifespan, it is imperative that the timing of insemination with FTS be optimized relative to ovulation. Most sows ovulate approximately 2/3 of way through estrus, but there is tremendous variation in the timing of ovulation relative to the onset of estrus. Therefore, methods of synchronizing estrus and ovulation have been investigated to improve FTS outcomes.

Sows experience strong inhibition to follicular growth and ovulation during lactation. The weaning event then removes the inhibition and naturally synchronizes follicular growth and ovulation in groups of sows. Gilts who have never been bred may require hormonal manipulations, such as progestogens, to control estrus. Estrus synchronization is typically achieved by exogenous hormones, most commonly Altrenogest, with the brand name of Matrix®, used in cycling sows and gilts. Altrenogest is an orally active progestogen that acts similar to progesterone's hormone action. When fed at a concentration of 2.2 mg/mL a day for the recommended 14 days, Altrenogest typically induces estrus 4 to 9 days after the last feeding. Altrenogest acts similar to progesterone by inhibiting GnRH release, preventing further follicular growth on the ovary (Flowers, 2001).

Altrenogest is used in estrus synchronization programs, where the last Matrix feeding (LMF) is used to time the subsequent insemination.

P.G.600® (Merck Animal Health, Madison, NJ) is a drug approved for use in gilts to stimulate puberty. However, this drug has also been evaluated in synchronization programs for stimulating follicular growth following weaning of sows. P.G.600® is administered as an injection, this allows for easy handling, and does not present similar hormone handling issues that Altrenogest may present. P.G.600® contains two hormones, eCG (equine chorionic gonadotropin) and hCG (human chorionic gonadotropin), that act to induce follicular growth and subsequent ovulation in gilts. In synchronization programs, P.G.600® tended to increase ovulation rate (P=0.07) in treated gilts compared to control gilts (Horsley et al., 2005). P.G.600® has the potential to improve the utilization of FTS, by synchronizing follicular growth post-weaning. In Spencer et al. (2010) P.G.600® was utilized in gilts as part of the fixed time artificial insemination program using FTS. It was found that P.G.600® after LMF reduced the duration of estrus, which could be beneficial for FTS utilization.

Despite controlling the timing of estrus, variation in the timing of ovulation relative to the onset of estrus still exists. Recently, advances in ovulation induction have been evaluated in conjunction with estrus synchronization, allowing for improved timing of insemination relative to ovulation. Commonly the induction of ovulation is performed with eCG or a GnRH agonist such as triptorelin acetate (OvuGel®, United Animal Health, Sheridan, IN). These hormones and agonists function by increasing LH and therefore inducing ovulation. OvuGel® is the only FDA approved drug for swine ovulation induction in the U.S.. It has been shown to stimulate ovulation 43.0 ± 1.5 h after administration (Kirkwood and Kaufford, 2015; Stewart et al., 2010) which provides an effective time period to breed post-weaned sows at 24 hours following administration of the product. Induced ovulation provides an effective avenue for development of single, fixed time artificial insemination (FTAI). OvuGel® provides a predictable time of ovulation, allowing for a single insemination to occur during the optimal window of time instead of having to inseminate females multiple times over the days of standing estrus. When paired with estrous synchronization, induction of ovulation has potential to improve fertility outcomes from FTS by optimizing the time of insemination relative to ovulation (Waberski et al., 1994).

1.5.3 Current Practices with FTS: Fixed Time Artificial Insemination

Single, fixed time artificial insemination (SFTAI) combines estrus and ovulation synchronization to improve conception rates with FTS. The goal of SFTAI is to decrease labor associated with estrus detection and conserve semen resources. Notably in the beef and dairy industries, SFTAI has been successfully implemented, however, it has yet to be widely adopted in the U.S. swine industry.

Previous FTS breeding protocols required labor intensive management practices, as demonstrated in Martin et al. (2000). This paper reported a breeding protocol where estrus detection was performed 3 times a day and gilts were inseminated 4 times every 6 hours with the first insemination being 30 hours after the first observation of estrus. This breeding protocol resulted in comparable conception rates of FTS to fresh, however the required labor resources were concluded to be ineffective in commercial swine operations. The goal of SFTAI is to capitalize on past knowledge of best timings to inseminate relative to estrus detection, ovulation timing, all in order to decrease labor requirements and semen doses.

In one notable study, Chanapiwat et al. (2014) demonstrated successful intrauterine inseminations of 2 x 10^9 billion cell FTS doses achieving 75% conception rates in multi-parous sows following estrus and ovulation synchronization compared to 88% conception rates using fresh semen at 3 x 10^9 cells (Chanapiwat et al., 2014). Fresh semen was found to result in higher total born, however the paper concluded that FTS with SFTAI is maximized when inducing ovulation and when estrus was observed. Spencer et al. (2010) performed estrous synchronization and ovulation induction and determined that the fertility was improved when gilts received their insemination at 24 and 36 hours after ovulation induction with an average induced ovulation occurring at 33 hours after onset of estrus. There was a sharp decrease in litter sizes when insemination to ovulation intervals were delayed, resulting in conception rates below 50% when ovulation occurred 48 h after onset of estrus. The success of ovulation induction relies on the presence of medium to large follicles on the ovary. Additional research into timing of insemination of FTS in relation to estrous synchronization and ovulation induction is warranted to improve fertility with FTS.

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CHAPTER 2. UTILIZATION OF FROZEN THAWED SEMEN IN LARGE BLACK PIGS

2.1 Abstract

The utilization of frozen thawed semen (FTS) in pigs has limited fertility evident in reduced conception rates and litter sizes in comparison to liquid semen storage options. Despite these limitations, semen freezing is the only long-term storage method which allows for elite sire preservation and long-distance shipping of genetics. Some minor breeds of swine are considered endangered such as the Large Black, and may benefit from a genetic importation of frozen semen. Thus a novel study was initiated, where sixteen Large Black nulliparious sows were selected and transported to Purdue University to undergo estrous and ovulation synchronization to improve FTS fertility utilizing frozen American Large Black semen in replicates 1-3 and imported United Kingdom Large Black semen in replicate 4. Following 14 days of Matrix feeding, OvuGel® was administered 144 h following last Matrix feeding (LMF) or 96 h in post-weaned sows with each replicate inseminating at two times following OvuGel®: 1 = 30 and 36 h, 2 = 17 and 23 h, 3 = 24 and 30 h, 4 = 24 and 32 h. Conception rates did not differ among replicates (1 = 25%, 2 = 30%, 3 = 68.8%, 4 = 50%; P=0.729). Follicle diameter (P=0.260) prior to OvuGel® administration nor ovulation status (P=0.411) influenced conception rate. Estrus expression at insemination was determined to significantly impact conception rate (P=0.043), finding that sows that conceived, 82.6% were in estrus and had a follicle diameter of 6.22±0.26 mm at OvuGel® administration. Parity 1 sows in replicate 3 had 3.4 fewer total born than parity 1 sows in replicate 1 and 3.0 fewer piglets than parity 1 sows in replicate 2 (P=0.022). A total of 75 piglets were weaned among replicates 1-3, with replicate 3 weaning significantly more piglets than both replicate 1 (P=0.049) and 2 (P=0.012). These results indicate that as the Large Black sows matured, FTS fertility was improved under intensive reproductive management practices improving the utilization of limited semen doses in a fixed time artificial insemination program.

2.2 Introduction

Frozen thawed semen (FTS) presents a great potential benefit of long-term storage options to the U.S. swine industry, however limited fertility associated with reduced conception rates and

litter sizes result in its lack of use (Johnson et al., 2000). Several assisted reproductive technologies (ART) have allowed for improved FTS fecundity, such as the utilization of intra-uterine insemination and estrous synchronization programs (Chanipawat et al., 2014). In the 1990s, it was determined that FTS has a limited post thaw lifespan and requires inseminations to occur only hours prior to ovulation in the sow (Waberski et al., 1994). However, more recent research using inseminations at 24 and 36 hours after onset of estrus have also shown acceptable fertility when using FTS (McNamara and Knox, 2013).

Proper estrus detection has been determined to be one of the limiting factors in AI for both liquid and frozen semen (Bolarin et al., 2006), however the U.S. swine industry is continuously developing methods to improve fertility and ease labor requirements. Fixed time artificial insemination (FTAI) practices are one method that may potentially decrease labor requirements and inaccurate estrus detection. FTAI poses a potential benefit for FTS fertility and utilization in the U.S. swine industry (Knox, 2016). However, past research has focused on FTS inseminations relative to onset of estrus, where few have studied FTS inseminations relative to ovulation induction (McNamara and Knox, 2013). Though FTS has had limited utilization in the U.S. commercial swine industry, small pork producers may potentially benefit from the advantages that AI presents in genetic diversity and reproductive management compared to natural service.

Small pork producers have been known to utilize minor swine breeds on their farms, also known as heritage swine breeds due to the historical context of these swine breeds. One particular heritage swine breed, the Large Black, is popular among niche pork producers due to its unique foraging and carcass traits, however this breed is critically endangered with less than 400 registered breeding individuals (Payne and Couch, 2020). In 2015, a collective of Large Black and Gloucestershire Old Spots breeders formed the heritage swine initiative to facilitate a genetic importation utilizing frozen semen from two boar lines not present in the U.S., however all insemination attempts failed in 2017 resulting in 0 liveborn piglets. Past research findings in other minor breeds have found reproductive differences in minor swine breeds (Egerszegi et al., 2003; White et al., 1995) such as age at puberty or reduced ovulatory responses, with there being little to no information on the Large Black. Additionally, the Large Black has experienced little to no genetic selection for traits of reproductive importance.

In order to determine the cause of this reproductive failure in heritage swine, the objective of this study was to determine the most efficient estrus and ovulation synchronization protocol to

efficiently utilize limited sperm numbers presented by high-value FTS. Therefore, it was hypothesized that the creation of a FTAI protocol for niche pork producers would be highly beneficial to improve the utilization of FTS allowing heritage swine breeders to capitalize on the benefit that FTS presents. Thus, this study utilized FTAI in combination with an estrus and ovulation synchronization program to facilitate the creation of piglets derived from the imported FTS.

2.3 Materials and Methods

2.3.1 Breeding Herd Characteristics and Animal Sourcing

The Purdue University Animal Care and Use Committee approved the synchronization and ovulation induction protocols utilized in this study (Protocol # 1801001688) performed in four breeding periods from January 2019 to February 2020. Twenty registered Large Black pigs were initially donated and transported to Purdue University Animal Sciences Research and Education Center (ASREC) Swine Farm in West Lafayette, IN sourced from nine individual purebred breeders across eight states detailed in Figure 2.1. Fifteen nulliparious and 1 multi-parious sows were included in this study representing Charlotte (n = 7), Daisy (n = 2), Matilda (n = 3), and Prudence (n = 4) dam lines. At animal transfer, donors transferred registry documentation to Purdue University. Following transport, animals were mixed and housed in isolation for disease monitoring from four to six weeks before being moved into Purdue University's swine farm. Animals were housed in groups in pens in an outdoor open-front building except during breeding and farrowing. Animals were limit fed a standard gestation (Table 2.1), according to body condition and body weight during gestation and fed ad libitum during lactation. Animals were periodically weighed to track growth. Animals that were identified with elongated toe nails received veterinary treatment which included sedation to allow the veterinarian to trim the toe nails.

Table 2.1. Purdue sow diet formulations, as fed basis

Ingredient, %	Lactation	Gestation
Corn	50.423	62.033
SBM 47% CP	31.500	12.850
DDGS - 7% fat	10.000	20.000
Swine Grease	3.000	1.000
Limestone	1.450	1.560
Monocalcium phosphate	1.310	0.510
Swine Vitamin Premix[1]	0.250	0.250
Sow Vitamin Premix[2]	0.250	0.250
Trace Mineral Premix[3]	0.175	0.175
Phytase[4]	0.100	0.100
Salt	0.500	0.500
Plasma protein	0.500	0.000
Citristim[5]	0.150	0.150
Availa Zn 120[6]	0.042	0.042
Clarify[7]	0.100	0.330
Defusion Plus[8]	0.250	0.250
Total	100.000	100.000
Calculated Nutrients		
ME, Kcal/kg	3340.60	3274.80
NE, Kcal/kg	2459.80	2453.00
CP, %	22.21	16.52
Total Lysine, %	1.185	0.720
SID Lys, %	1.001	0.550
SID Met, %	0.308	0.255
SID Met+Cys, %	0.614	0.494
SID Thr, %	0.694	0.475
SID Tryp, %	0.231	0.132
SID Iso, %	0.800	0.533
SID Val, %	0.888	0.643
Ca, %	0.900	0.754
Total Phos., %	0.725	0.521
Avail. Phos., %	0.498	0.351

[1]Provided per kilogram of the diet: vitamin A, 6,615 IU; vitamin D3, 662 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22 mg; niacin, 33 mg; vitamin B12, 38.6 mg.
[2]Provided per kilogram of the diet: vitamin E, 22.05 IU; biotin, 0.22 mg; folic acid, 1.65 mg; choline, 551.25 mg; pyridoxine, 4.96 mg; chromium, 0.20 mg; carnitine, 49.61 mg.
[3]Provided available minerals per kilogram of the diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg, Se, 0.30 mg.
[4]Provided 600 FTU of phytase per kg of the diet (Phyzyme, Danisco Animal Nutrition/DuPont, St. Louis, MO)
[5]CitriStim (ADM Animal Nutrition, Quincy, IL) is a proprietary strain of *Pichia guilliermondi*, a whole-cell inactivated yeast product.
[6]Availa Zn 120 (12% Zn chelate premix; Zinpro Corporation, Eden Prairie, MN Zinpro Corp.)
[7]Clarifly (Central Life Sciences, Schaumburg, IL) provided 6.7 and 22.1 ppm diflubenzuron as a larvicide in the diet when included at 0.10, and 0.33%.
[8]Defusion Plus (Provimi, Brookville, OH) a blend of feed preservatives and other ingredients.

Figure 2.1. Approximate locations of donor farms. Public domain image from Blank_US_map_borders_labels.svg

2.3.2 Donor Surveys and Relations

Donors were voluntarily surveyed on various breed and management practices. Six out of 9 participating donors returned answered surveys. Questions were divided into 4 sections comprised of general farm questions, breeding, farrowing, and products with a final question asking what the donor wished to receive from the Purdue breeding project. The survey was conducted from March 25th to April 15th, 2019. Survey questions can be found in Appendix A.

Prior to animal transport and pickup, each donor signed a contract with the Livestock Conservancy that transferred animal ownership to Purdue University. Thank you cards to each of the donors were sent out with invitations to a private Facebook group allowing for consistent contact among donors and research progress. Originally, the Facebook group was to serve as a quarterly newsletter, however pictures and updates were continuous and consistent with breeding, farrowing, and finishing events. Following project completion, the Facebook group is intended to serve as a breeder discussion platform facilitating the coordinated dispersal and management of piglets from the United Kingdom imported semen

On June 26th, 2019, the Large Black Swine Workshop was held at Purdue University hosted by the USDA National Animal Germplasm Preservation Group (NAGP), University of Missouri, and Purdue University. Out of the 34 Gloucestershire Old Spots and Large Black breeder stakeholders, 11 breeders were in attendance at Purdue University. The workshop began plans to draft a breed wide implementation plan and suggested to the breeders a Heritage Swine Association. In September 2019, the Large Black Hog Association (LBHA) leadership began plans

to draft a contract for dispersal of American and UK genetics offspring born at Purdue. By May 2020, 8 initial Large Black donors had signed the contractual cooperative agreement sponsored by the Livestock Conservancy for imported semen derived piglet dispersals. Copies of signed contractual agreements were shared by LBHA leadership with Purdue University and the Livestock Conservancy.

2.4 Breeding, Gestation and Farrowing Management

2.4.1 Breeding Methodologies

To meet the objectives of this project, animals were bred multiple times over a two-year period to first determine the best protocols to optimize fertility using FTS followed by utilization of high-value imported semen to create offspring of novel genetics for the U.S. The first three breeding replicates were conducted with frozen semen sourced from American Large Black boars. Each breeding replicate was altered based on Large Black reproductive performance to determine the best methods for improving Large Black conception rates and farrowing rates in the next subsequent breeding replicate in preparation for utilizing imported semen. Reproductive measures such as standing estrus and preovulatory follicle size were considered variables in the creation of the breeding protocols. Table 2.2 describes the total number of sows synchronized per breeding replicate and frozen semen sources. Breeding replicate 4 was conducted utilizing imported United Kingdom Large Black semen from a genetic import into the United States. Replicate 4 breeding protocol was conducted based on previous reproductive performance measures to improve utilization of limited straws of imported semen.

Table 2.2. Overview of breeding replicates

Replicate	Number of sows	Boar genetics
1	16	US
2	10	US
3	16	US
4	10	UK

One donated animal aborted an unknown pregnancy following transition into Purdue University’s farm. Two donated animals were determined to be prepubertal and were not included

in breeding replicate 1 and 2 until puberty was documented prior to breeding replicate 3. Animals were treated with Draxxin (Zoetis USA, Parsippany, NJ) following veterinary diagnoses of mycoplasma pneumonia prior to replicate 1. All animals were treated for sun-burning with Flunixin prior to breeding replicate 2.

2.4.2 Estrous Synchronization and Ovulation Induction

Synchronization and insemination was performed in 4 replicates during the 2019-2020 year with breed dates taking place during months of different seasons: replicate 1 = February, replicate 2 = April, replicate 3 = September, replicate 4 = January. All 4 breeding replicates followed an individual fixed timed artificial insemination protocol with slight modifications based on previous reproductive performance and conception rates. Fence-line boar contact and back pressure estrus detection was performed to determine cyclicity in all animals due to unknown reproductive records prior to breeding replicate 1. The number of sows and boars utilized per breeding replicate are described in Table 2.3.

Prior to breeding replicate 1, 87.5% (14/16) were determined to be cycling as determined by estrus detection. Estrus was synchronized using Matrix® (Merck Animal Health, Summit, NJ) and ovulation induced using OvuGel® (United Animal Health LLC; Sheridan, IN). Matrix® was administered orally 7.0 mL (Altrenogest 2.2 mg/mL, Merck Animal Health) for 14 d by placing a dosing syringe in the corner of the gilt's mouth and delivering the drug to the back of the throat. OvuGel® was administered between 144 h (replicates 1, 3, and 4) or 154 h (replicate 2) after the last Matrix® feeding. Replicate 2 last Matrix® feeding was delayed due to delayed follicle growth observed in replicate 1 as described in Figure 2.2. OvuGel® contains the drug triptorelin acetate administered in a 200 mg bolus in the cranial portion of the vagina using the applicator gun provided by the manufacturer. Two artificial inseminations (AI) with frozen semen were performed following OvuGel® administration. The time from OvuGel® administration to insemination varied slightly among the breeding replicates where: replicate 1 = AI at 30 and 36 hours after OvuGel®, replicate 2 = AI at 17 and 23 hours after OvuGel®, replicate 3 = AI at 24 and 30 hours after OvuGel®, replicate 4 = AI at 24 and 32 hours after OvuGel®.

In breeding replicates 3 and 4 some sows had just weaned a litter from the previous breeding replicate. These animals had estrus synchronized by the weaning event at 0800 h followed by OvuGel® administration 96 hours after weaning (Figure 2.2). Two artificial inseminations were

performed at the same times following OvuGel® as described above. Following the last Matrix® feeding, estrus detection was performed twice daily at 0800 h and 1600 h by back-pressure test with fence-line exposure using sexually mature crossbred boars with the exception of replicate 1 where estrus detection was performed only once daily.

Ultrasound was used to visualize the animal's ovaries in order to measure the size of the follicles on the ovary prior to initiation of synchronization and to confirm ovulation events following AI. For replicates 1 and 2, ovarian ultrasound was performed abdominally using a SonoSite Micromaxx machine with a 5 mm convex rC60xi/5-2 MHz probe (Fujifilm SonoSite Inc., Bothell, WA). For these procedures, the ultrasound probe was covered in ultrasound gel and the probe was placed in the animal's flank. The bladder was used as a reference point to locate the uterine tissue. Ovaries were then identified within the uterine tissue. During replicates 3 and 4, ultrasound was performed transrectally using a 5 mm convex rC60xi/5-2 MHz probe on the same ultrasound machine. The linear probe was placed inside a custom-made plastic handle. The handle and probe were lubricated prior to insertion into the rectum of the female. The same landmarks were used to locate and identify the ovaries. Prior to synchronization, the diameter of the largest two follicles on the animal's ovary were measured using software included on the ultrasound machine. Approximately 44-48 hours after OvuGel® administration, ultrasound was performed to determine whether ovulation occurred. The animals were scored based on ovulation status where the presence of corpora lutea (CL) on the ovary was classified as ovulated, and the presence of preovulatory follicles was classified as not ovulated

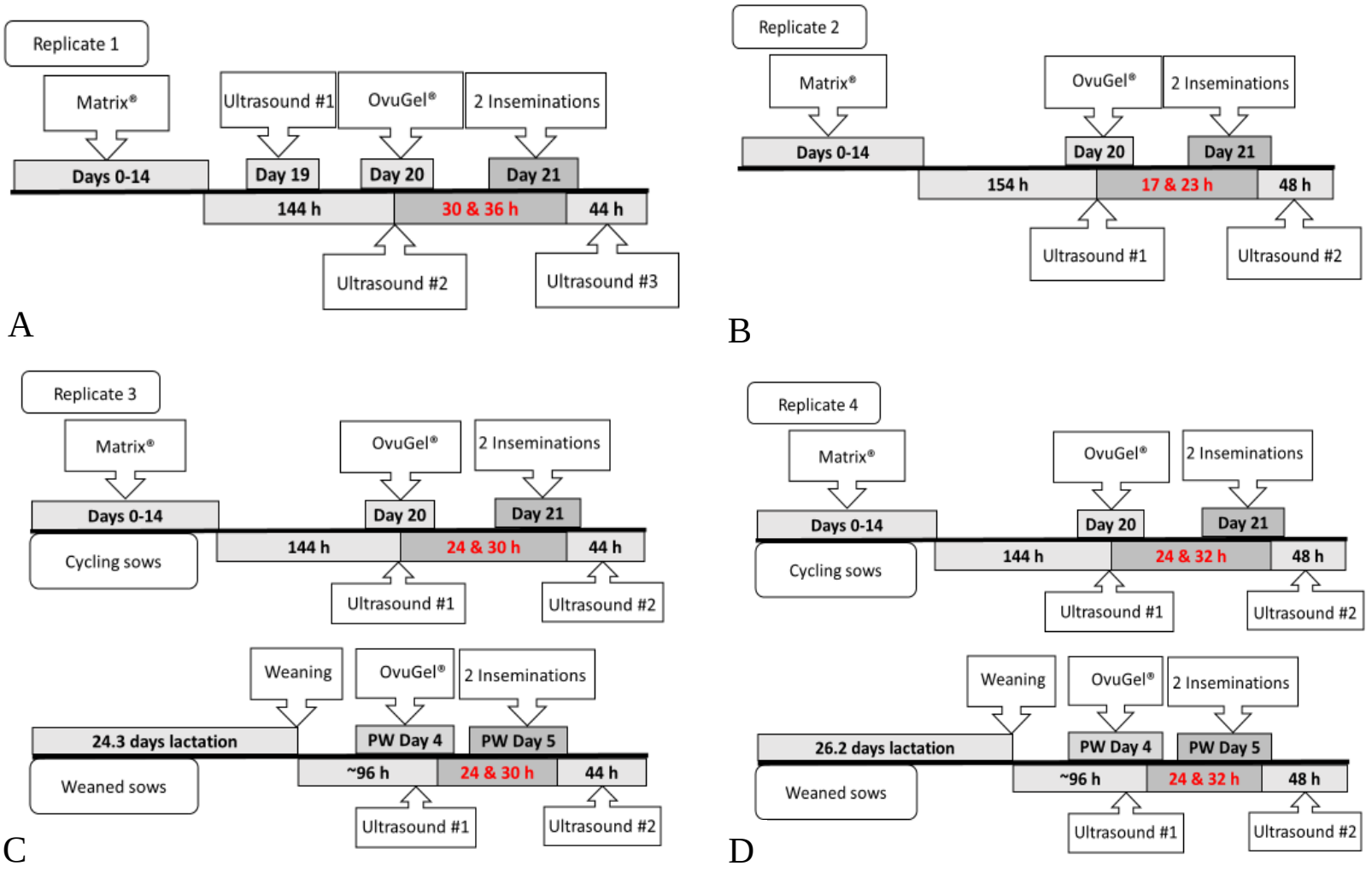

Figure 2.2. This figure describes the synchronization protocols and timings of insemination that each breeding replicate followed. Figure A, B, C, and D followed: 30 and 36 h, 17 and 23 h, 24 and 30 h, and 24 and 32 h, respectively. Figures C and D included post-weaned sows (PW) that were synchronized alongside nulliparious cycling sows.

2.4.3 Semen Thawing and Preparation

Information about the US Large Black boar semen shipped from International Boar Semen (IBS) is described in Table 2.4. For replicates 1-3, guidance on semen thawing procedures was provided by IBS. According to IBS protocols, doses with 75% or greater motility and less than 25% abnormal cells were frozen. Straws were packaged to have between 5 to 6.25 billion total cells. The 5 mL straws were removed from the dry shipper and exposed to room temperature air for 7 seconds then submerged into a warmed water bath at 42 °C for 43 seconds to thaw the semen. The straw was then cut and the thawed semen was placed into 40 mL of warmed (37 °C) Androhep Plus (Minitube USA; Verona, WI) semen extender. These 40 mL doses were immediately used for artificial insemination. Post-thaw motility in breeding replicates 1 and 2 were analyzed on farm by warming a droplet of the extended semen sample on a slide warmer at 37 °C for 10 minutes and viewing semen under a bright-field microscope. In breeding replicate 3, motility was evaluated using a computer-assisted semen analysis (CASA, CEROS II™, Hamilton Thorne, Beverly, MA) from one straw per sire (n=5 boars). Concentration was measured in breeding replicates 1 and 2 using a SpermaQ Photometer (Minitube USA; Verona, WI).

Table 2.3. Number of US boars and straws

Replicate	Number of sows	Number of boars	Number of straws
1	16	6	32
2	10	3	20
3	16	5	32

Table 2.4. US Large Black boar information

Boar	Name	Farrow Date	Origin	Line	Replicates
9901	Little Buck 9901[1]	-	IL	-	3
9920	SM Noble Sam 6/1 #148	5/14/2003	MS	Noble Sam	1
9921	SM Majestic 3/1 #79	3/5/2002	MS	Majestic	1, 2, 3
9922	Sunrise Phillip 261N 1453476	9/2/2003	ON	Super	1, 2
9923	Sunrise Phillip 292N	9/5/2003	ON	Super	3
9934	Large Black Commercial 9934[1]	-	-	-	3
9958	Cornish Majestic 7/3 0837	11/11/2010	MO	Majestic	1, 2, 3
9980	Walnut Grove Majestic 1/8 3254	2/12/2013	KS	Majestic	1
9981	Dogwood Hills Longfellow 5/1 2249	5/12/2012	AR	Longfellow	1

[1]Boars without registered LBHA pedigrees

For replicate 4, frozen semen from two Large Black boars each representing sire lines not currently present in the United States (Malcolm and Super) were imported according to USDA APHIS protocols. UK Large Black boar registry and origin information is described in Table 2.5. The boars came from Deerpark Pedigree Pigs (Bellaghy, Magherafelt, Northern Ireland). Semen was collected and packaged into 52 straws from each boar with two insemination doses per 2.5 mL straw in June of 2015. Deerpark Pedigree Pigs did not share its freezing protocol with the USDA ARS NAGP. The imported semen was shipped to USDA ARS NAGP and stored in liquid nitrogen dewars. Prior to breeding replicate 4, 5 straws per boar were overnighted in a dry vapor shipper to Purdue University for storage until use.

The USDA NAGP procedures for thawing 5 mL straws were used for the imported 2.5 mL straws. Straws were removed from liquid nitrogen and submerged in water at 50 °C for 50 seconds. The straws were cut open and the thawed semen placed into 80 mL of warmed (37 °C) semen extender (Androstar Premium, Minitube USA, Verona, WI). Sperm cells were mixed into the 80 mL extender, then the sample was divided into two 40 mL insemination doses. Ten doses were randomly assigned to 10 sows for insemination. One mL was removed from the extended semen, warmed for 10 minutes at 37 °C, and evaluated for motility using phase contrast microscopy. Following motility, samples were preserved with 10% formalin and concentration was determined using a Nucleocounter (Reproductive Provisions LLC, Walworth, WI).

Table 2.5. UK Large Black boar information

Boar	Name[1]	Farrow Date	Origin	Line
KBB/61	Donagheragh Malcolm 61 6-6 R002180LB	12/4/2012	Northern Ireland	Malcolm
KBB/93	Donagheragh Super 93 6-6 R002312LB	10/28/2013	Northern Ireland	Super

[1]British Pig Association pedigrees.

2.4.4 Artificial Insemination (AI)

Sows were housed in gestation crates for several days prior to and following artificial insemination. Breed registries were checked to determine the relationship of the sire to the dam and matings were determined to minimize inbreeding prior to thawing. Post-cervical artificial insemination (PCAI) catheters (Minitube USA, Verona, WI) were placed into the reproductive tracts of animals regardless of estrus status. Thawed semen was passed through the catheter into

the female's reproductive tract immediately after thawing and extending. The second insemination was performed in the same manner as the first using the same sire as the first insemination. Each insemination was scored based on the ease of the semen dose entering the female and amount fluid loss during insemination where: 3 = semen entered female with ease and no fluid loss, 2 = slight challenges with semen entry and small amounts of semen loss, and 1 = challenging to get semen into female and large amounts of semen lost. Presence of blood on the catheter was recorded in addition to any issues with placing the catheter into the sow's uterus.

2.4.5 Pregnancy Determination

Live ultrasound scans were used to determine pregnancy status. Abdominal ultrasounds were performed 28-29 days after the date of insemination. For replicates 1-3, ultrasound scans were performed using a SonoSite Micromaxx machine (Fuijifilm SonoSite Inc., Bothell, WA) with a 5 mm convex probe covered in ultrasound gel placed in the animal's flank then gradually pointed cranially towards the head. The bladder was used as a reference point to locate the uterus and presence of fluid filled sacs, indicating presence of embryos. Presence of at least one fluid filled sac that were dark in color and round in shape indicated positive pregnancy status. Lack of dark shapes and presence of only uterine tissue indicated a negative pregnancy status. For replicate 4, ultrasound scans were performed using a wireless ultrasound SV-2 scanner (Veterinary Sales and Service, Stuart, FL) following the same external flank ultrasound methodology. Animals were then moved out of breeding gestational stalls to the outdoor open-front building during gestation periods.

2.4.6 Gestation Management

After pregnancy diagnosis, animals were housed in 1 of 4 pens in an outdoor open-front building with straw provided as bedding. Stocking density was typically 3 to 4 animals per pen of similar body weight and size. All reasonable attempts were made to maintain social groups during the entire project to minimize stress from fighting. Animals were observed frequently by researchers to track weight and health care management. Animals were often socialized with humans providing treats in the form of marshmallows and apple juice. Animals were checked for general health and alertness, and identified for any lameness issues requiring therapeutic treatments.

Animals were limit fed a standard gestation diet (Table 2.1) that met or exceeded NRC (2012) nutrient requirements, and fed according to body condition and body weight size. Gestation feed was provided by drop box feeders with a pulley system. Animals were observed and fed daily by Purdue University staff and pens were frequently scraped and manure was placed in a designated compost pile. Clean straw was often eaten and routinely added to bed each pen. Water was provided ad libitum in each pen by nipple waterers.

2.4.7 Farrowing Management

Animals that conceived and carried to full term, were moved into standard farrowing crates by day 112 of gestation. Sows were fed ad libitum a standard lactation diet (Table 2.1) which met or exceeded NRC nutrient requirements (NRC, 2012). Farrowing crates were a bow bar design with 0.55 m x 2.22 m of sow space. Animals were induced to farrow using 2 mL of Lutalyse® (10 mg dinoprost) at 1000 h on day 114 of gestation. Purdue University Swine Farm farrowing protocols were followed with obstetrical assistance when needed. Farrowings were attended recording birth weights, time of birth, number of pulled pigs, assistance given and sex of the offspring. Piglets were removed from sows that exhibited signs of aggression and savaging for a period of time and kept under a heat lamp. Piglets were weighed 24 hours after recorded time of birth for 24 hour weights. Piglets were given 1 mL of iron (100 mg/mL) at processing at 3 days of age, and an additional 1 cc at weaning. Ear punches were collected from piglets sired by registered boars and shipped to USDA NAGP in 1 mL tubes containing glucose media for genotyping. All piglets with pedigrees were issued litter certificates registering the litter with the LBHA and USDA NAGP for genotyping. Lactation length averaged 24.3 days in replicates 1 (n = 3 litters) and 2 (n = 3 litters) and 26.4 days in replicate 3 (n = 9 litters).

During replicate 3, sows were weighed and ultrasound scanned for backfat changes during early lactation and as well as the day before weaning. Before ultrasound scanning, sows were palpated for last rib and tenth rib locations. An Aloka 500V ultrasound probe (Aloka Co., Ltd., Tokyo, Japan) was used. The Aloka 3.5 MHz probe (Aloka Co., Ltd., Tokyo, Japan) was placed at the last rib to measure back fat. The same probe was placed at 10th rib to measure backfat and loin depth. Loin muscle area was difficult to accurately measure due to the large size and thick backfat of the sows. Sows were weighed on the day of ultrasound to calculate weight loss during lactation on a certified floor scale.

2.4.8 Statistical Analysis

All data were analyzed using SAS v9.3 (Cary, NC). Conception rate was analyzed with the GLIMMIX procedures using binary distribution and logit link function. The model included the fixed effects of breeding replicate, while parity status, estrus status at breeding, follicle diameter class (≤ 5, $5 < 6$, ≥ 6), confirmed ovulation, and inbreeding class were used as covariates. All other parameters were analyzed using the MIXED procedures with multiple comparisons of means using the Tukey Kramer adjustment. The model included the fixed effects of breeding replicate, parity of the dam and genetic line of the dam, while boar and inbreeding class (0-10%, > 10%) were used as covariates. Due to time constraints, only replicate 1-3 are included in farrowing data analysis, with replicate 4 included in the breeding data analysis. Data are reported as LS Means. Statistical significance was determined to be $P<0.05$, with tendencies being $0.05 < P \leq 0.10$.

2.5 Results

2.5.1 Breeding Herd Characteristics and Animal Sourcing

Twenty sows entered Purdue University, however only 16 animals were utilized among all breeding replicates 1-4. All Large Blacks were diagnosed with mycoplasma pneumonia and provided veterinary care prior to replicate 1, with (n = 4) sows being removed due to health reasons and structural conformation issues from the study prior to breeding replicate 2. The overall average inbreeding coefficient for all sows (n = 20) was 14.1±1.6%, with a range of 7.5% to 33.3%. Prior to breeding replicate 1, it was determined that not all Large Blacks (n = 16) were nulliparous (n = 15), with 1 multiparous included in this study. The mean age of the animals upon arrival at Purdue University was 347.0±31.9 days of age (n = 20). The average age of sows at breeding were: replicate 1 = 464.4±35.1 days, replicate 2 = 520.4±44.4 days, replicate 3 = 635.2±38.3 days, replicate 4 = 793.7±38.9 days. The initial mean body weight was 141.5±9.9 kg, with a final mean body weight of 221.7±6.9 kg, which indicates that the Large Black sows (n = 16) matured in body weight during the course of this study with an average daily gain of 0.21±0.2 kg between January 2019 and January 2020.

Sows that were ultrasound scanned for backfat at early lactation (n = 9) had an average body weight of 225.2±15.3 kg. These sows had an average weight loss of 6.94±1.41 kg over the course of 20 days, resulting in the mean body weight at weaning of 219.9±7.11 kg. Early lactation

last rib backfat was determined to be 4.8±0.45 cm and 10th rib backfat was 4.4±0.24 cm. At weaning the last rib backfat was determined to be 4.6±0.4 cm and 10th rib was determined to be 4.6±0.4 cm. Loin depth and loin eye area was determined to be difficult to accurately measure due to size of animal and thickness of the backfat.

2.5.2 Estrus Synchronization, Ovulation Induction, and Conception Results

From all 4 replicates, 52 matings were performed using these 16 animals where 16 were bred for replicate 1, 10 for replicate 2, 16 for replicate 3, and 10 for replicate 4. The results for estrous synchronization and ovulation are in Table 2.6. Conception rates for each breeding replicate were: 1 = 25.0%, 2 = 30.0%, 3 = 68.8%, and 4 = 50.0%. Prior to OvuGel® administration follicle diameters were categorized as < 5 mm, 5-6 mm and > 6 mm and averaged for each breeding replicate: 1 = 4.66±0.19 mm, 2 = 5.68±0.28 mm, 3 = 6.52±0.11 mm, and 4 = 6.42±0.17 mm. Inbreeding coefficients were categorized as 0-10% and >10% and averaged for each breeding replicate:1 = 11.83±0.73%, 2 = 11.88±0.89%, 3 = 14.69±1.93%, and 4 = 11.71±0.98%. Body weight prior to starting each replicate breeding protocol averaged: 1 = 141.5±9.9 kg, 2 = 177.1±15.1 kg, 3 = 204.0±10.5, and 4 = 221.7±6.9 kg.

Approximately 2.64±0.3 billion motile sperm cells were utilized in each 5 mL straw extended to 40 mL insemination dose in replicates 1-3 (Table 2.7). Mean post-thaw motility per boar is found in Tables 2.7. and 2.8. The Deerpark Pedigree Pigs' 2.5 mL straws were calculated to contain 2.06±0.12 billion total cells per straw resulting in an average of 0.34±0.03 billion motile sperm cells per insemination in replicate 4. In replicate 4, 2 sows received a full 2.5 mL dose extended to a single 40 mL insemination at 32 hours after OvuGel® containing 0.47±0.25 billion motile sperm cells. One of these two sows conceived. Scoring of insemination and presence of blood on the catheter were considered insignificant covariates not included (P>0.10).

Conception rate did not differ among the replicates (P=0.729) or parity (P=0.348). Sows that were in estrus at the time of insemination were more likely to conceive (P=0.043). Follicle diameter class at OvuGel® administration (P=0.260) nor ovulation within 48 hours of OvuGel® (P=0.411) had significant effects on conception rate. Body weight (P=0.681) and inbreeding class (P=0.213) also did not affect conception rate. When estrus at AI was analyzed as the dependent variable, there was no significant replicate (P=0.880), parity (P=0.322), replicate by parity effect (P=0.541), or preovulatory class (P=0.938) effect. Of the sows that conceived, 82.6% (19/23) were

in estrus, had a preovulatory follicle diameter of 6.22±0.26 mm, and 81.0% (17/23) ovulated within 48 hours of OvuGel® administration. In sows that did not conceive, 55.2% (16/29) were in estrus, had a preovulatory follicle diameter of 5.42±0.23mm, and 76.9% (20/29) ovulated within 48 hours of OvuGel® administration.

2.5.3 Farrowing

All of the farrowing data can be found in Table 2.9. Farrowing rates were 100% with the exception of replicate 1, which yielded a farrowing rate of 75.0%. In replicate 1, one confirmed pregnant sow aborted 6 piglets at 62 days of gestation. During the farrowing process, 35.0% (n = 6) of sows across replicates 1-3 were observed to display aggressive behaviors towards newborn piglets in the first 24 hours, resulting in the direct loss of 5 piglets and removal of piglets for a period of time during the farrowing process. Other causes of preweaning mortality included double splay legs and crushing, likely due to low viability piglets.

The average induced gestation length for each replicate was: 1 = 115.3, 2 =114.0, 3 = 116.8 days in length. Of all induced Large Blacks, 58.8% (10/17) farrowed on day 115 following induction on day 114. Only 11.8% (2/17) sows farrowed prior to or by day 114 and 29.4% (5/17) sows farrowed after day 115. One sow in replicate 3 did not farrow until 125 days of gestation and she only had 2 non-viable piglets, which were euthanized shortly after farrowing.

All 6 sows that farrowed in replicates 1 and 2, also farrowed in replicate 3 classified as parity 2 sows. Replicate 3 had fewer total piglets (P=0.010) and born alive (P=0.026) compared to replicates 1 and 2 (Table 2.9). Across all 3 replicates, parity 2 sows tended to have larger litter sizes than parity 1 sows (P=0.066). Parity 1 sows in replicate 3 had 3.4 fewer total born than parity 1 sows in replicate 1 and 3.0 fewer piglets than parity 1 sows in replicate 2 (P=0.022). Replicate 3 parity 1 sows tended to have 2.9 fewer total born (P=0.079), and 2.4 number born alive (P=0.111) than parity 2 sows (6.5±1.0 vs 3.6±1.0). Sow lines tended to be different (P=0.052), with Prudence having more total born than Charlotte. Sire also influenced total born (P=0.039) and tended to influence number born alive (P=0.058). IBS boar 9921 sired the most piglets on average with 5.6 piglets per litter sired. Replicate 3 parity 2 sows had larger nursing litter sizes (P=0.019) than parity 1 sows by 3.6 piglets (5.8±0.86 vs 2.2±0.94). There was no significant replicate (P=0.353), parity (P=0.431), line (P=0.636), inbreeding class (P=0.418), or sire (P=0.916) effects on number of stillbirths. Replicate 3 tended to have fewer mummies compared to replicate 1 (P=0.084). Line

(*P*=0.198), inbreeding class (*P*=0.416), and sire (*P*=0.572) did not influence number of mummies. There were no significant effects on number of boars (*P*=0.198) or gilts (*P*=0.219) born alive. Average birth weight was not different among the replicates (*P*=0.139), but there was a tendency for parity to influence average birth weight (*P*=0.089) with replicate 3 parity 2 sows having 0.06 kg heavier piglets than parity 1 sows. There was a tendency for average 24 h weights to differ (*P*=0.072), with replicate 3 having greater 24 h weights than replicate 2. Across all 3 replicates, a total of 75 piglets were weaned with replicate 1 = 13 piglets, 2 = 18, and 3 = 44 piglets. There was a significant replicate effect on number of pigs weaned (*P*=0.015), with replicate 3 weaning more piglets than both replicate 1 (*P*=0.049) and replicate 2 (*P*=0.012). Daisy sows farrowed only in replicate 3 and failed to wean piglets resulting in 0 piglets weaned (*P*=0.007); Prudence sows weaned 1.98 kg heavier piglets than Charlotte (*P*=0.007). Replicate 3 parity 2 sows tended to wean heavier litters (*P*=0.092) than replicate 3 parity 1 sows.

2.5.4 Piglet Dispersals

All replicate 1 and 2 piglets were weaned and went through nursery and finishing at Purdue University for carcass data (Chapter 3). However, following replicate 3 farrowing, piglets were determined to be returned to Large Black donors. A total of 44 piglets were weaned, 10 of which were registered with the LBHA having both sire and dam registry records. A total of 42 piglets were returned to donor farms. At about 6 weeks of age, 42 piglets were transported with certificates of veterinary inspection, health records, and pedigrees by 3 donors to 6 farms located in Colorado, Illinois, Indiana, Ohio, and Pennsylvania from Purdue University. Three boar piglets from replicate 2 and 1 boar piglet from replicate 3 farrowing were transported to IBS in Eldora, Iowa.

2.6 Discussion

The objective of this study was to determine the most efficient synchronization and breeding protocol to maximize fertility using high-value FTS in a heritage breed of swine. Due to reduced fertility of FTS in swine, the ability to successfully synchronize females and utilize reduced numbers of viable sperm cells is necessary. Most research with FTS in swine uses around 1 billion motile cells to inseminate (Spencer et al., 2010; Knox and Yantis, 2014). In this study, the high-value, imported semen was a limiting factor. Therefore, successful synchronization and

timing of insemination relative to ovulation was critical for success. This study was confounded on multiple levels with age of the animals and previous reproductive performance between replicates. Additionally, health challenges early in the study could have impacted the early replicate results. However, some general interpretations can still be made from the data.

The synchronization program utilizing Matrix and OvuGel® resulted in 50-80% of the females in heat at the time of insemination and 60-80% of the animals ovulating by 48 hours after OvuGel®. Replicate 1 had the lowest number of animals in heat at AI which could be due to the fact that the animals were younger at replicate 1 as well as health challenged from mycoplasma. This replicate also included all animals that were synchronized with Matrix and did not include any animals that were bred following a weaning event, as did replicate 2 which followed 2 months later. Overall, conception rate in animals that were synchronized with Matrix had a 40.0% (16/40) conception rate compared to post-weaned sows with 58.3% (7/12). The animals that did not conceive in replicate 1 were rebred in replicate 2 and 3, so the Matrix-fed group of females could be less fertile as they were not random in replicate 2 and 3. However, when considering selection of animals for a program such as this where high-value semen is to be utilized, it may be best to consider utilization of post-weaned sows as compared to younger, potentially less fertile, and unproven animals. Replicate 4 was comprised of mostly weaned sows (9/10) that had conceived previously in the study (i.e. did not receive Matrix®, only OvuGel®) and resulted in 88.9% in heat at AI and 77.8% ovulating within 48 hours of OvuGel®.

The expression of estrus at artificial insemination was the only variable that significantly impacted conception rate where 54.3% (19/35) of the animals in heat at AI conceived compared to only 23.5% (4/17) of the animals that were not in heat at AI conceived. When utilizing limited high value semen, inseminating without the onset of estrus or ultrasonography is not recommended. Accurate estrus detection is an important determining factor in artificial insemination programs (Flowers et al., 1992), as it is indicative of a female with large enough follicles on her ovary to produce high levels of estrogen prior to ovulation. Once the estrogen levels reach threshold concentrations, they induce the LH surge and ovulation. In this study, 64.7% (11/17) of animals not in estrus at AI still ovulated by 48 hours after OvuGel® compared to 74.2% (26/35) of the animals that were in estrus at AI. Therefore, being in estrus at AI increased the likelihood of ovulating within 48 hours by 19% and increased conception rates by 30%. As reported in Stewart et al. (2010), OvuGel® synchronized gilts were found to ovulate at 43.8$\pm$1.5 hours, which is likely

the case in the Large Black with ovulation rates at 44-48 hours to be greater than 50%. In the current study it was not determined when ovulation occurs in relation to first observed of estrus, only confirmed that ovulation occurred within 48 hours of OvuGel®. When using OvuGel® to synchronize ovulation, it is expected that some animals will not show estrus, but will still ovulate and conceive as OvuGel® will induce ovulation of follicles on the ovary. This was observed in this study where 64.7% (11/17) animals did not express estrus, yet ovulated by 48 hours after OvuGel® and of these, 4 conceived (4/17), 3 of which ovulated within 48 hours. These animals would not have received semen if the AI was based solely on estrus status. Therefore, inducing ovulation proved beneficial to the synchronization program to increase the likelihood of conception of animals not displaying signs of estrus.

Due to a limited sample size (n = 16 animals and 52 matings), it is challenging to find significant differences in binomial variables such as conception rates. Though statistical differences were not seen among replicates, numerical differences suggest that insemination times of 24 and 30-32 hours after OvuGel® administration is appropriate for synchronized Large Black sows to establish conception rates that result in live born piglets (i.e. 50.0-68.8% seen in replicates 3 and 4). In replicate 2 where insemination occurred at 17 and 23 hours, 80% of the sows were in heat at insemination and 60% ovulated by 48 hours after OvuGel®, indicating successful estrous synchronization, however, conception rates were lower than other replicates (30%). This suggests that insemination times of 17 and 23 hours after OvuGel® was not optimal to achieve adequate conception rates likely due to semen being deposited into the females too far ahead of ovulation. According to Waberski et al., (1994) insemination with fresh semen is optimal up to 12-8 hours before ovulation, and is reduced to 4 hours with frozen semen in order to produce a conception rate comparable to fresh semen. However, other studies have shown success of FTS when the insemination-to-ovulation interval is increased up to 12 or more hours (McNamara and Knox, 2013), where a greater proportion of piglets were sired by the first insemination, furthest from ovulation when using post-thaw motility greater than 26%. When using OvuGel®, ovulation occurs 43.8±1.5 hours after administration of the drug (Kirkwood and Kaufford, 2015; Stewart et al, 2010). In the present study, time of ovulation was not determined, only a single evaluation of whether ovulation had occurred within 48 hours of OvuGel®. Therefore, the exact insemination-to-ovulation interval cannot be determined, only estimated. So, relative to timing of ovulation, replicate 1 inseminations were 13 and 7 hours ahead of predicted ovulation, replicate 2 = 26 and

20 hours, replicate 3 =19 and 13, and replicate 4 = 19 and 11. In theory, replicate 1 insemination timings would have occurred closer to predicted ovulation in support of the Waberski et al. (1994) optimal timing where as the other replicates would have been more in line with McNamara and Knox (2013). Since Waberski et al. (1994), advancements in frozen semen handling and storage have likely improved the lifespan of FTS in combination with assisted reproductive technologies (ART).

In the current study, replicate 1 had the lowest conception rate with only 25% of sows conceiving. With the confounding in this study, it is impossible to determine whether this lowered conception rate was due to timing of inseminations or maturity of the animals used as only 50% of the animals in replicate one were in estrus at the time of AI and all were gilts. Animals in replicate 1 were approximately 15 months of age and 141.6 kg. Sexual maturity in the Large Black typically occurs at or over 7 months of age, however maturity is influenced by body weight and the degree of boar exposure the gilt has received. In the 1940s, Large Black gilts were estimated to reach sexual maturity at 219.2±1.7 days (Burger, 1952), which is likely similar to today's Large Black gilts. In the current study, it can be assumed that the gilts were pubertal for a period of time based on the estrus detection rates that improved once the gilts began to mature in age. What can be observed in the current study is that inseminations at 17 and 23 hours post-OvuGel® as used in replicate 2 (26 and 20 hour insemination-to-ovulation interval) was likely too early since 80% of the animals were in estrus at AI yet only 30% conceived. Additional animals and research is needed to evaluate the differences in timings of inseminations when using FTS. In a situation where semen is of high-value, if the timing of the inseminations relative to ovulation could be optimized, then there is potential for a single insemination. However, in order for this to be successful, sows would likely need to be proven as fertile (i.e., post-weaned sow) and in estrus at the time of AI.

Nine out of the 10 animals in replicate 4 were post-weaned sows which resulted in 88.9% (8/9) in heat at AI without Matrix feeding and 77.8% (7/9) ovulating by 48 hours after OvuGel®. This replicate had a reduced conception rate of only 50.0%. This is likely due to the reduced sperm numbers used to inseminate. Replicates 1-3 utilized approximately 2.6 billion motile sperm cells compared to only 300 million in replicate 4. This is considered a large difference in the number of motile sperm and would be expected to impact fertility. However, when using high-value semen, it is challenging to determine how to maximize conception using the least quantity of semen. Literature using SFTAI and PCAI with fresh semen from 1.2 billion to 75 million cells has shown

acceptable pregnancy rates (70-80%) with reduced litter sizes (Knox et al., 2019). Knowing that frozen semen has a reduced lifespan and fertility compared to fresh, it is expected to perform at a reduced fertility as in replicates 1-3 compared to fresh semen. This study supports that idea where conception rates were lower than what would be expected with fresh semen if 2.6 billion sperm cells were used. In actuality, achieving a 50% conception rate with 300 million motile sperm cells from FTS could be considered quite successful. At the time of writing this manuscript, replicate 4 had not farrowed, so interpretation of low sperm numbers on litter sizes are not possible at this time.

Litter sizes were numerically lower in replicate 3 compared to 1 and 2, however there was quite a large amount of variation in litter sizes ranging from 3-11 piglets. It is not known what the Large Black average litter size is to compare, however minor swine breeds and outdoor reared sows are thought to have smaller litter sizes and wean less pigs per sow than major swine breeds (Picardy et al., 2017; Ratky et al., 2013). The litter sizes observed in the current study are definitely lower than is observed in commercial breeds which were observed to farrow litter sizes of 13.4 in 2012 (Kraeling and Webel, 2015). In replicate 3, the parity 1 females that farrowed had the lowest litter sizes and they accounted for 5/11 farrowings in this group. The parity 1 females in replicate 3 averaged 3.6 total born vs the parity 2 sows that averaged 6.5. Replicate 1 and 2 only had parity 1 females that farrowed and the average total born was 7.0 and 6.7, respectively. The parity 1 females in replicate 3 then were the animals that did not conceive in replicates 1 and 2 and, therefore, could be considered less fertile.

This project has been impacted by several confounding factors such as health status and age, however, it can be considered a successful genetic import resulting in live-born piglets from imported frozen semen in a rare heritage breed of swine. It appears as that as the Large Black pig ages and matures, her fertility increases. While at Purdue, the animals were fed a nutrient dense diet, similar to those seen in commercial swine production, which allowed the Large Black females to grow rapidly in body mass reaching their likely potential mature body mass which may have contributed to enhanced reproductive success over time. At the end of this study, the Large Black sows were considered obese at 221 kg. Overweight sows typically have reduced longevity and are more expensive to maintain (Kim et al., 2016). Large Black sows have been reported to reach weights of up to 363.0 kg (Livestock Conservancy, 2020), thus the weights that were achieved in

this study were within the Large Black's potential mature body mass, but may have attained the weight quickly with a large amount of fat deposition.

Based on this study a recommended synchronization protocol can be found in Figure 2.3 utilizing several assisted reproductive technologies, Matrix®, OvuGel®, and PCAI to improve FTS conception rates. This synchronization protocol for both cycling and weaned sows requires the female to be in estrus prior to FTS insemination 24 and 32 hours after OvuGel® administration. When utilizing ultrasound imagery, a follicle size of greater than 6 mm (i.e. 6.22±0.26 mm) is recommended prior to OvuGel® administration. If the sow is not observed to be in estrus prior to FTS, it is recommended to administer OvuGel® when estrus is first observed then inseminate at 24 and 32 hours following administration. This synchronization protocol has not yet been tested on farm by Large Black breeders, and it is not currently known if similar results can be achieved on-farm. Differing management styles and experience in AI may be influential in the success of an on-farm synchronization and insemination protocol. This on farm protocol may require a relationship with an extension specialist or educator to ensure on farm handling of the synchronization protocol is executed accordingly.

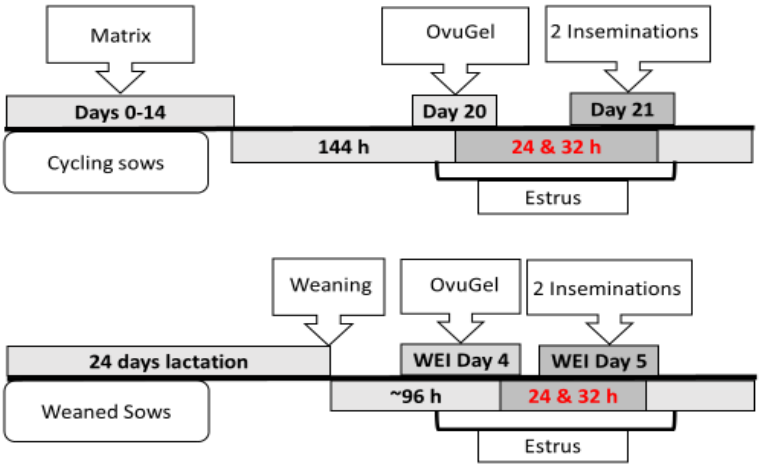

Figure 2.3. Recommended synchronization breeding protocol. Note that the sow must be in estrus prior to insemination, however may not be in estrus at OvuGel® administration

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Table 2.6. Synchronization responses and fertility results

Item	Replicate 1: 30 & 36 h	Replicate 2: 17 & 23 h	Replicate 3: 24 & 30 h	Replicate 4: 24 & 32 h
Estrus Rate, %	50.0% (8/16)	80.0% (8/10)	68.8% (11/16)	80.0% (8/10)
Ovulation Rate, %	73.3% (11/15)	75.0% (6/8)	80.0% (12/15)	88.9% (8/9)
Conception Rate, %	25.0% (4/16)	30.0% (3/10)	68.8% (11/16)	50.0% (5/10)
Weaned Sow Conception Rate, %	-	-	100% (3/3)	44.4% (4/9)
Matrix Conception Rate, %	25.0% (4/16)	30.0% (3/10)	61.5% (8/13)	100.0% (1/1)
In Estrus and Ovulated, %	62.5% (5/8)	62.5% (5/8)	81.8% (9/11)	87.5% (7/8)
In Estrus and Conceived, %	37.5% (3/8)	37.5 (3/8)	81.8% (9/11)	50.0% (4/8)
Ovulated without Estrus, %	75.0% (6/8)	50.0% (1/2)	60.0% (3/5)	50.0% (1/2)

Table 2.7. US Large Black post-thaw semen evaluation

Replicate	Boar ID	Number of Straws	Concentration (millions per mL)	Dose Volume (mL)	Total Cells (billions)	Average Motility (%)	Total Motile Cells per straw (billions)	Number of Conceptions
1	9920	4	0.1278	40	5.11	26	1.34	0
1	9921	6	0.1982	40	7.93	47	3.70	2
1	9922	6	0.1782	40	7.13	28	2.02	1
1	9958	6	0.1525	40	6.10	38	2.34	1
1	9980	6	0.1888	40	7.55	49	3.71	0
1	9981	4	0.1885	40	7.54	26	1.98	0
2	9921	4	0.2093	40	8.37	40	3.35	1
2	9922	8	0.1753	40	7.01	41	2.89	1
2	9958	8	0.1218	40	4.87	50	2.44	1
3	9901	10	-	40	-	25	-	5
3	9921	4	-	40	-	30	-	1
3	9923	4	-	40	-	15	-	2
3	9934	8	-	40	-	20	-	3
3	9958	6	-	40	-	34	-	0

[1]Total and total motile sperm cells were averaged per straw, in which one straw equaled one dose.

Table 2.8. UK Large Black post-thaw semen evaluation

Replicate	Boar ID	Number of Straws	Concentration (millions per mL)	Dose Volume (mL)	Total Cells[1] (billions)	Motility (%)	Total Motile Cells per dose[2] (billion)	Number of Conceptions
4	UK Malcolm	5	0.025	40	2.33	34	0.39	3
4	UK Super	5	0.025	40	1.79	28	0.24	2

[1]Total cells was calculated per straw.

[2] Total motile cells were calculated as one straw divided by two doses as packaged by Deerpark Pedigree Pigs.

Table 2.9. Large Black farrowing results

	Replicate				Statistical Significance, $P<$				
Item	1	2	3	SEM	Replicate	Parity	Line	Inbreeding Class	Sire
Total born, pigs	7.00	6.67	5.18	1.15	0.010	0.007	0.052	0.860	0.040
Number born alive, pigs	6.00	6.33	4.91	0.74	0.026	0.017	0.103	0.830	0.058
Stillbirths, pigs	0.67	0.33	0.09	0.67	0.353	0.431	0.636	0.418	0.916
Mummies, pigs	0.33	0.00	0.18	0.33	0.087	0.025	0.198	0.416	0.572
Boars born alive, pigs	3.00	3.00	2.55	0.58	0.198	0.277	0.343	0.710	0.154
Gilts born alive, pigs	3.00	3.33	2.27	0.88	0.220	0.135	0.314	0.352	0.262
Average birth weight, kg	1.57	1.47	1.66	0.24	0.139	0.089	0.236	0.329	0.407
Average 24 hr weight, kg	1.83	1.56	1.97	0.24	0.072	0.107	0.304	0.437	0.169
Number weaned, pigs	4.33	6.00	4.00	1.00	0.015	0.007	0.068	0.685	0.054
Average weaning weight, kg	6.92	6.28	8.04	1.03	0.291	0.877	0.007	0.203	0.673

CHAPTER 3. GROWTH AND CARCASS CHARACTERISTICS OF LARGE BLACK PIGS FED DIETS SUPPLEMENTED WITH OR WITHOUT ALFALFA

3.1 Abstract

Genetic selection has increased growth rates, lean growth, and reproductive efficiency in major swine breeds increasing the profitability of pork production. One minor swine breed, the Large Black (LB) is attractive to pastured pork production and marketed based on its pork quality traits, however has relatively no information regarding these traits compared to commercial breeds. A 2 x 2 factorial design experiment evaluating Large Black (LB) and Duroc sired (DS) pigs fed corn based diet (CON) or corn based diet supplemented with alfalfa and wheat middlings (FIB) was carried out to study the effects of genotype and diet on growth and carcass characteristics in 25 LB and 40 DS pigs similar in age under commercial conditions. Following 6 phases of grow-finish diets, pigs were harvested for digestive organs and carcass data. Carcass measurements were carried out 24 hours post-mortem. Loin muscles were evaluated for fresh pork quality and instrumental color and tenderness. LB pigs had a reduced rate of gain (ADG) than DS pigs (P<0.0001) and G:F (P<0.0001). Pigs fed FIB had reduced ADG (P=0.020), and poorer G:F (P=0.007). LB pigs had lighter whole visceral masses (P<0.0001), liver (P<0.0001), kidney (P<0.0001), and empty intestinal weights (P=0.010) than DS pigs. Pigs fed FIB tended to be lighter at slaughter (P=0.070), have lighter total intestinal weights (P=0.015), however as a proportion of liveweight, the FIB pigs had heavier whole visceral masses (P=0.005). LB pigs were 26.3 kg lighter (P<0.0001), 28.5±1.3 cm^2 smaller longissimus muscle area (P<0.0001), and yielded 2.0 cm more 10^{th} rib back fat than DS pigs (P<0.0001). However, the LB pigs had lower loin drip loss (P=0.009), and cooked loin shear force (P<0.0001). These results indicate that the LB pig is slow growing, and a fibrous diet did not significantly slow the accretion of fat in the LB pig. The FIB diet increased percent lean, and resulted in lighter body weights reached at slaughter, which is consistent with past research findings studying fibrous materials in non-ruminant nutrition.

3.2 Introduction

Advances in assisted reproductive technologies have increased genetic selection for traits of economic importance, decreasing days to market, increasing pounds of lean muscle produced, and increased litter sizes. Improvements not only in reproductive and genomic technologies, but also advancements in nutrition and environmental control have increased productivity for swine producers resulting in an increase in the amount of pork produced with less animals (Kraeling and Webel, 2015). Through genetic selection for these traits of economic importance, the U.S. swine industry has relied on 6 major swine breeds that are able to be marketed to produce lean pork products at 6 months of age. The 6 breeds are Yorkshire, Duroc, Berkshire, Hampshire, Landrace, and Chester White. Crossbreeding of these 6 major swine breeds have allowed for the swine industry to capitalize on heterosis, allowing for highly heritable growth and carcass traits, but also lowly heritable reproductive traits to maximize the increased productivity of pigs.

As pork production in the U.S. becomes a more integrated system, the number of large farms has increased while the number of small farms has decreased (USDA NASS, 2019). Therefore, alternative marketing strategies have been developed to help the small farms stay in business. However, there is a movement in a portion of the U.S. swine industry to market pork based on trait attributes or social credences that are different than commercial pork producers (Honeyman et al., 2006). The niche pork production system emerged from changes in consumer taste and preferences, however remains a minor part of the U.S. industry. Examples of niche pork marketing is “organic”, “pasture raised” and “heritage”. Through niche pork production practices, small pork producers have incorporated minor swine breeds, otherwise known as heritage breeds, into their swine operations to produce and market pork. Heritage swine breeds are attractive to niche pork producers, of which most are considered endangered. One such heritage swine breed, the Large Black is considered critically endangered, however growing in popularity among non-traditional pork producers due to the Large Black pig’s foraging behavior and carcass qualities (Livestock Conservancy, 2020). Similar to other minor swine breeds, the Large Black pig became undesirable to pork processors due to lack of lean growth and extended scalding processes for their dark hair coats, as well as lack of consumer interest in lard pork products (Dohner, 2001; Porter et al., 2016).

Attractive to niche pork production systems, the Large Black is commonly found in pasture-based farms consuming a variety of feedstuffs. Niche pork producers have been encouraged to

feed pigs various feedstuffs (Talbott et al., 2003), however a pasture diet would be high in fibrous materials and may not completely meet the nutritional requirements of swine. Past research has studied the effects of low energy diets on growth and carcass characteristics, and found that low net energy diets reduce body weights but increase lean muscle (Schinckel et al., 2015). Diets with low energy may reduce lipid accretion in pigs, however this also comes at the expense of lean growth (De Greef and Verstegen, 1995). Despite the popularity to niche producers, there is limited research comparing growth and carcass characteristics of pasture-raised to conventionally-raised pigs (Edwards, 2003). Research on swine reared in outdoor environments is difficult to control due to the effects of variables such as season, forage quality, supplementation, and management practices which all could impact growth rates (Juska et al., 2013; Hoffman et al., 2003). It has been estimated that in order for pasture pork producers to remain financially stable, a market premium must counteract the disadvantages outdoor production and various genotypes present (Kelly et al., 2007). Niche pork producers rely on pasture to provide a significant portion of the animal's nutritional requirements, however it is not known how this influences the growth of a lard type pig such as the Large Black.

There is a lack of information that characterizes the purebred Large Black's growth and carcass qualities in comparison to today's commercial pork breeds. In order to capture these differences, the Large Black pig must be studied under controlled research conditions to understand the effects of genotype and diet. Therefore, the objective of this study was to determine the effects of a high forage diet (corn-based diet supplemented with and without alfalfa and wheat middlings) on growth and carcass qualities in the Large Black pig in comparison to commercial crossbred pigs.

3.3 Materials and Methods

3.3.1 Animals and Treatments

This study is a 2 x 2 factorial design evaluating genotype (Large Black (LB) vs Duroc-sired (DS)) and diet fed Control or Fiber on growth and carcass traits. This study was conducted in two replicates based on two farrowing groups of purebred American Large Black pigs. At approximately 63.8 days of age, 25 LB pigs and 40 DS, age-matched pigs were allotted into mixed sex pens based on genotype, sex, and initial body weight. DS pigs were crossbred pigs from Duroc

semen inseminated to Yorkshire x Landrace dams. Replicate 1 had 11 LB and 20 DS pigs resulting in 4 pens for CON and 4 pens for FIB, two for each genotype. Replicate 2 had 14 LB and 20 DS pigs resulting in 4 pens for CON and 4 pens for FIB, two for each genotype. Pens were 1.83 m x 2.43 m each with a single hole self-feeder and nipple waterer on concrete slatted floors. Animals were observed daily for wellbeing by trained staff. Feeders were checked daily for feed clogs, and flow rate was adjusted to minimize feed wastage.

Pigs were fed either a control diet composed of corn, soybean meal and DDGS (CON) or a high fiber diet composed of wheat middlings and dehydrated alfalfa (FIB) (Table 3.1). Both diets were formulated to meet or exceed nutrient requirements according to NRC (2012). The diets were phase fed over 18 weeks where the composition of the diet was adjusted every 21 days, resulting in 6 phases of feeding. Diet compositions for each phase and treatment are described in Table 3.1. Feed was sampled at each phase change and frozen at -20 °C for future feed analysis.

3.3.2 Feed Intake and Body Weight

Prior to study initiation, all pigs were weighed using a certified floor scale (Rice Lake Weighing Systems, Rice Lake, WI) and again at the end of each sequential diet phase change. Feed was delivered ad libitum via Farmweld Stainless Steel Feeder® (Farmweld Inc., Teutopolis, IL), and amounts delivered to each feeder were recorded to calculate feed intake per pen. At the end of each diet phase, all feeders were weighed on IQ 390 DC platform scale (Rice Lake Weighing Systems, Rice Lake, WI) and 25 lbs or less of feed was left in the feeder to transition to the next diet phase. Body weight and feed intake were used to calculate Average Daily Gain (ADG), Average Daily Feed Intake (ADFI), and Gain to Feed (G:F) for each pen.

3.3.3 Growth Measurements

At three time points (days 42, 84 and end of study) ultrasonography was used to measure backfat thickness, loin depth and loin muscle area. Cross section ultrasonography was performed using an Aloka 500V ultrasound (Aloka Co., Ltd., Tokyo, Japan) fitted with a 3.5 MHz probe (Aloka Co., Ltd., Tokyo, Japan). Before ultrasounding, animals were hand palpated for determination of last rib and 10^{th} rib locations. Backfat depth at the last rib and 10^{th} rib were measured using software on the ultrasound machine. Loin depth and loin muscle area were

measured at the 10th rib. Measurements were obtained on 2 pigs per pen (1 barrow and 1 gilt) that represented the pen's average weight at the beginning of the study.

Heart girth measurements were collected by wrapping a cloth measuring tape around all Large Black pig's chest just behind the shoulders. Heart girth measurements were taken following final live weight measurements to calculate a regression equation prior to slaughter. Heart girth data were used in the Groesbeck et al., (2002) equation which is used to predict live weights of pigs. The equation is:

$$(\text{Liveweight kg } \widehat{Y} = 4.6127(\text{Heartgirth cm}) - 93.31)$$

3.3.4 Slaughter

At the end of diet phase 6 (replicate 1= 185.5 ± 0.41 days of age, replicate 2= 203.4 ± 0.36 days of age), pigs were individually tattooed and transported in two loads, heavy and light blocks, to the state inspected Purdue University abattoir. Feeders were removed the evening prior to slaughter to decrease gut-fill. Pigs were electrically stunned followed by exsanguination. Pigs were then scalded and dehaired. After head removal, whole visceral mass and leaf fat were removed and weighed prior to carcass splitting. Liver and kidneys were separated from the whole visceral mass, and weighed separate from the visceral mass. Percent of live and carcass weight was determined as each visceral organ/mass divided by body weight*100. A subset of 10 pigs per dietary treatment had their gastrointestinal tracts (stomach to anus) cleaned and washed out with a spray hose nozzle. After dissection and washing, empty intestinal tracts were weighed without the mesentery and spleen. Total empty intestinal tracts were weighed including the mesentery and spleen on a certified Fairbanks scale (Fairbanks Scales, St. Johnsburg, VT). Female reproductive tracts were evaluated and scored as being mature or prepubertal based on the presence of corpora lutea or corpora albicans indicating maturity. The carcass was split at the midline and hot carcass weights of both halves were recorded on a Rice Lake© Rail Scale (Rice Lake Weighing Systems, Rice Lake, WI). Carcasses were immediately placed into a 4 °C cooler for 24 hours prior to carcass evaluations the following day.

3.3.5 Carcass Measurements

Carcass length was measured starting at the first rib ending at the aitch bone found in the pelvis using a measuring tape. Backfat was measured at three midline locations: first and last rib, and last lumbar vertebrae prior to carcass ribbing. The left side of the carcass was ribbed for carcass data collection between the 10th and 11th rib. Ribbed carcass fat depth, loin muscle area, and hot carcass weight were used to calculate percent lean and fat free lean (Schinckel et al., 2001). A cross section of the 10th rib loin muscle were traced on clear trans-parent plastic and measured using the Iowa State Extension and Outreach plastic grid (AS-235e, Iowa State University, Ames, IA).

3.3.6 Wholesale Cuts

The right side of the carcass was broken down for untrimmed wholesale cut weights, weighing jowl, neck bones, picnic, Boston butt, loin, spare ribs, ham, and belly weighed on a Rice Lake© model IO+355 2A scale (Rice Lake Weighing Systems, Rice Lake, WI).

3.3.7 NPPC Standards and Scores

After allowing the ribbed carcass loin muscle to bloom for 20 min, color, firmness and marbling scores were measured following NPPC standards by a trained technician (NPPC, 2000). Color scores were scored with 1 being pale pink to 6 being dark red in color. Marbling scores were determined by 1 being devoid to 10 being abundant. Firmness was scored with 1 being very soft to 5 being very firm.

3.3.8 Color and 24 hour pH

Two measurements of instrumental color were recorded by Minolta CR-400 Chroma Meter (Konica Minolta, Tokyo, Japan) per carcass loin muscle following wholesale cut breakdown recording L* (lightness), a* (redness), and b* (yellowness). A pH probe (HANNA HI 99163, Hanna Instrument, Inc., Warner, NH) was inserted directly into the loin muscle at the 11th rib side of the 10th and 11th rib interface at two sites per ribbed carcass and averaged for 24 hour post-mortem pH.

3.3.9 Cooking Loss, Drip Loss, and Shear Force

Individual samples were cut from each pig's loin muscle from 10th to the 13th rib for proximate analysis, drip loss, and Warner Bratzler Shear Force (WBSF) measurements. For proximate analysis, the loin was cut at 1.27 cm and a 2.54 cm strip of backfat was removed and packaged for later analysis. Two 1.27 cm pork chops were weighed prior to placement and suspension in an expanded half zipped 3.7 L Ziploc bag then placed in 4 °C for 24 hours. After 24 hours the chops and purge were weighed and averaged to determine drip loss. WBSF pork chops (2.54 cm) were vacuum sealed in 3-mil vacuum seal bags and placed in a 4 °C cooler for 7 days until analysis. Prior to cooking WBSF samples were weighed and then cooked on an open face fryer (Model GR-150, Cuisinart, Stamford, CT) to a final internal temperature of 71 °C. Cooking loss was calculated from WBSF samples, by weighing before and after cooking. After cooking, WBSF samples were chilled in 4 °C overnight then cored in 1 x 1 cm sections parallel to muscle fibers using a Warner Bratzler type V-shaped blade to measure instrumental tenderness (Stable Micro System Ltd., Surrey, UK). The location of the LB cores were less selective than DS cores due to their reduced loin muscle areas.

3.4 Statistical Analysis

All data were analyzed using SAS v9.3 (Cary, NC). All growth measurements were analyzed with pen as the experimental unit (n=16). All growth parameters were analyzed using the MIXED procedures. The model included fixed effects of replicate, genetics, dietary treatment, body weight block, and appropriate interactions. All carcass parameters were examined using the MIXED procedures with animal as experimental unit (n = 50). The model included fixed effects of replicate, genetics, dietary treatment, and sex, with appropriate interactions. Data are reported as LS Means. Values of $P \leq 0.05$ were considered significant and $0.05 \leq P \leq 0.10$ as tendencies.

3.5 Results

3.5.1 Animals and Treatments

Replicate 1 pigs started the trial at 89.7±0.4 days of age, which was 9 days into diet phase 2 of finishing and consumed diets for 101 days. Replicate 2 pigs started the trial at 65.3±0.4 days of age and were on trial for 140 days completing all 6 diet phases. During the course of this study,

one replicate 2 DS pig died of unknown causes and is included in pen estimates until phase 4. One Large Black barrow developed an inguinal hernia, and was exchanged for another ultrasound pig, however is included in growth and carcass data.

3.5.2 Feed Intake and Body Weight

Average initial start weight across replicates was 25.9 kg and was not different between diet and genetics ($P<0.688$). LB pigs grew slower during every dietary phase ($P<0.001$), resulting in the LB pigs being 25.1 kg lighter at market (Table 3.2; $P<0.001$). However, ADFI was not different between genetics at any time period ($P>0.135$), leading to DS pigs being more feed efficient (G:F) in every phase (except phase 6) and overall grow-finish period ($P<0.001$). Feeding the FIB diet tended ($P<0.080$) reduced ADG during phases 3, 4, and 5 leading to an overall ADG reduction ($P<0.020$) that resulted in FIB fed pigs being 8.7 kg lighter at market ($P<0.045$). FIB fed pigs were less feed efficient (G:F) in phases 3, 4, 5, 6 ($P<0.050$), and overall ($P<0.007$).There were no diet by genetic interactions ($P>0.090$) at any time point throughout the growth portion of the study (Table 3.2).

3.5.3 Growth Measurements

DS pigs were heavier at all three scan dates (Table 3.3; $P<0.0001$). DS pigs had significantly larger LEA and deeper loin depths than LB pigs at all three ultrasound time points ($P<0.0001$). DS pigs had significantly decreased 10^{th} rib backfat ($P<0.0001$) at all three time points. These differences led to the DS pigs having greater calculated FFL and percent lean than the LB pigs at market weight ($P<0.0001$).

Feeding a high fiber diet reduced market weights of the scan pigs ($P<0.036$), however there was a diet by genetics interaction for 10^{th} rib backfat at market ($P<0.028$), with FIB pigs having about 1 mm more fat for the DS pigs but 10 mm less backfat in the LB pigs. Gilts were leaner at market last rib and 10^{th} rib backfat at d 84 and market ($P<0.032$) leading to gilts having a higher percent lean ($P<0.036$).

LB heart girth data was used to predict liveweights at slaughter with the Groesbeck et al. (2002) equation resulting in predicted values that were 13.1±0.01% greater than the observed liveweight values. LB heart girth for barrows and gilts was correlated with live weight at slaughter

(r^2 = 0.92 and 0.85, Figure 3.1). Since barrows were heavier than gilts at slaughter, two equations were calculated categorized with a 95% confidence interval: $\text{Gilts kg } \hat{Y} = 1.5923(\text{Heartgirth cm}) - 80.751$; $\text{Barrows kg } \hat{Y} = 1.9034(\text{Heartgirth cm}) - 117.9$ (Figure 3.1). One gilt that weighed <80 kg was removed after identifying as an outlier using Cook's Distance. The average residual difference between observed and predicted liveweights were 0.036±1.08 kg.

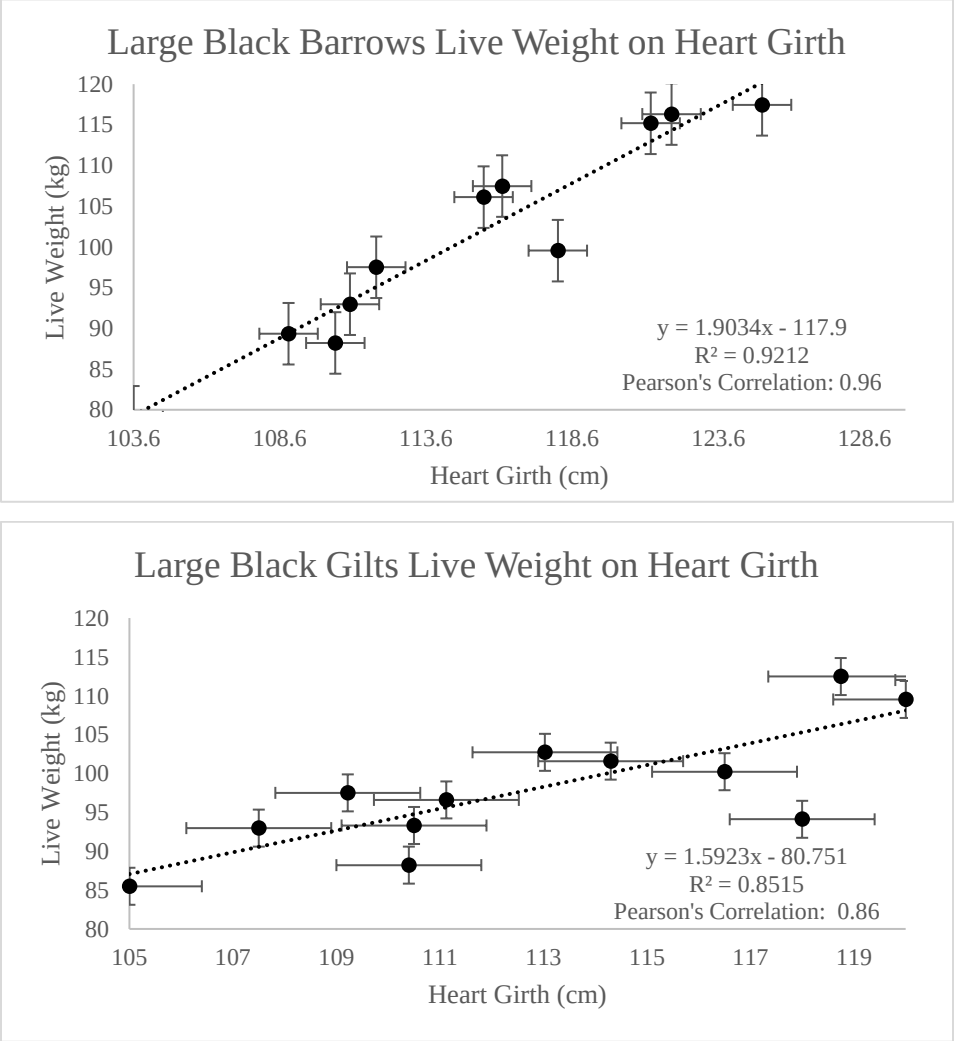

Figure 3.1. Large Black Barrows and Gilts Live Weight (kg) on Heart Girth (cm)

3.5.4 Slaughter

All slaughter data can be found in Table 3.4. One LB gilt was found to have a kidney abscess at slaughter, therefore was included in growth data but removed from carcass data. LB

pigs were 26.4 kg lighter at slaughter (P<0.0001) than DS pigs. LB pigs had lighter visceral organ mass (P<0.0001) than DS pigs. LB pigs had 24.5 kg lighter empty body weights (EBW, P<0.0001) than DS pigs. There were no genetics by diet interactions for live weight (P<0.090). DS pigs had heavier liver weights (P<0.0001) and kidney weights (P<0.0001) than that of LB pigs. DS pigs had heavier empty intestinal weights (P=0.010), and total GI weights (P=0.0051) than LB pigs. LB pigs tended to have greater empty intestinal mass relative to carcass weight (P=0.094) than DS pigs. When adjusted for carcass weight, LB pigs had a greater proportion of viscera (P<0.0001) and leaf fat (P<0.0001). Almost all DS gilts (85%; 11/13) were sexually mature, in comparison to only 14% (2/14) of LB gilts (P=0.021).

There was a significant diet effect on liveweight (P=0.002) for CON pigs to be 8.3 kg heavier than FIB pigs. There was a treatment by sex interaction, with CON gilts having larger kidneys (P=0.021) than CON fed barrows. CON barrows had heavier kidneys (P=0.008) than FIB barrows. CON pigs had heavier total GI weights (P=0.015) than FIB pigs. When visceral mass was calculated as a percent of live weight, pigs fed FIB had 1.03% heavier visceral mass proportion of live weight (P=0.005) than that of pigs fed CON and 1.8% greater when calculated relative to carcass weight (P=0.002). Liver weight as a percent of live weight (P=0.039) and relative to carcass weight (P=0.004) was greater in FIB pigs than CON pigs.

Barrows were found to have more leaf fat than gilts (P=0.0003). Gilts had a greater liver mass relative to live weight (P=0.049) than barrows. Replicate 1 pigs had heavier liver weights (P=0.0002) and kidneys (P=0.019) than replicate 2 pigs.

3.5.5 Carcass Measurements

Carcass characteristics are described in Table 3.5. DS pigs had heavier hot carcass weights (P<0.0001) than LB pigs by approximately 22.5 kg and cold carcass weights (P<0.0001). DS pigs yielded higher dressing percentages (P<0.0001) than LB pigs by 2.37%. LB pigs had shorter carcass lengths (P<0.0001) than DS pigs by approximately 5.12 cm. LB pigs had significantly greater first rib backfat (P=0.0001) than that of DS pigs, by approximately 0.91 cm. LB pigs had 1.2 cm more last lumbar backfat (P<0.0001) than DS pigs. LB pigs had greater last rib backfat (P=0.001) than DS pigs by approximately 1.3 cm. LB pigs had 2.0 cm more tenth rib backfat (P<0.0001) than DS pigs. DS pigs had 2.9 cm deeper loin depths (P<0.0001) than LB pigs. LB pigs had significantly smaller Loin Muscle Area (LEA, P<0.0001) than DS pigs by approximately

28.5 cm^2. DS pigs produced 24.6 more kg of fat free lean (P<0.0001) than LB pigs, resulting in 16.6% more percent lean than LB pigs (P<0.0001).

CON pigs had heavier hot carcass weights (P=0.003) than FIB pigs by approximately 8.5 kg as well as cold carcass weights (P=0.003). CON pigs had higher dressing percentages (P=0.0002) than FIB pigs. CON pigs had more first rib backfat (P=0.003) than FIB pigs by 0.67 cm. CON pigs had more last lumbar backfat (P=0.011) than FIB pigs. CON pigs had 0.46 cm more tenth rib backfat (P=0.017) than FIB pigs. CON pigs had more kg of fat free lean (P=0.042) than FIB pigs, however FIB pigs had greater percent lean (P=0.021).

Barrows yielded greater backfat depositions than gilts, evident in first rib backfat (P=0.005), 10^{th} rib backfat (P<0.0001), and last rib backfat (P=0.054). There was a significant genetics by sex interaction (P=0.020) where LB gilts had 0.5 cm deeper loin depths than LB barrows and DS barrows had 0.1 cm deeper loin depths than DS gilts. Gilts had tended to have larger LEA than barrows (P=0.054). There was a significant genetics by sex interaction (P=0.012) where LB gilts had 5 kg more FFL than LB barrows while DS barrows had 0.3 kg more FFL than DS gilts. There was an observed genetics by sex interaction (P=0.004) where LB gilts were 7.1% more lean than LB barrows, and DS gilts were 2.4% greater percent lean than DS barrows.

3.5.6 Wholesale Cuts

Untrimmed wholesale cut results are described in Table 3.6. Boston butts were approximately 1.6 kg lighter (P<0.0001) in LB pigs than DS pigs. DS pigs had 1.2 kg heavier (P<0.0001) picnic shoulders than LB pigs. DS pigs also had heavier hams (P<0.0001), loins (P<0.0001), neckbones (P=0.005), and spareribs (P<0.0001) than LB pigs. DS pigs had 0.86 kg heavier bellies (P=0.012).

FIB fed pigs had lighter jowls than CON fed pigs (P<0.0001). There was a diet by genetics interaction (P<0.0001), with DS pigs having heavier jowls but FIB fed LB pigs having significantly lighter jowl weights than LB pigs fed CON. CON pigs had heavier loins (P<0.0001), larger spareribs (P=0.003), and heavier bellies (P=0.014) than FIB pigs.

Barrows had heavier jowls (P=0.010) and larger neckbones (P=0.011) than gilts. Gilts had heavier loins (P=0.026) than barrows. There was an observed replicate effect, finding that replicate 1 pigs had heavier picnics (P=0.010) and hams (P=0.0006), as well as larger spareribs (P=0.0033)

than replicate 2 pigs. Replicate 2 pigs had heavier jowls (P=0.003) and larger neckbones (P<0.0001) than replicate 1 pigs.

3.5.7 NPPC Standards and Scores

LB pigs scored darker loin muscle using the NPPC color scores compared to DS pigs (3.3 vs 2.9, P=0.006), had higher marbling scores (3.1 vs 2.5, P=0.002), and had higher firmness scores (P<0.0001) than DS pigs (Table 3.7). Diets had no effect on fresh loin muscle characteristics (P>0.437). Replicate 1 pigs had higher marbling scores than replicate 2 pigs (P=0.004).

3.5.8 Color and 24 hour pH

Minolta instrumental color assessment and 24 hour pH values can be found in Table 3.8. LB pigs had a higher 24 hour post-mortem pH than DS pigs (5.63 vs. 5.60, P=0.016). Replicate 1 pigs had a higher 24 hour post-mortem pH than replicate 2 pigs (5.63 vs 5.60, P=0.012). LB pigs tended to have less b* than DS pigs (P<0.090). There was a diet by sex interaction, for barrows fed CON to contain less L*, darker loin colors (P=0.081) than barrows fed FIB. Replicate 1 pigs had more b star (P<0.0001) than replicate 2 pigs.

3.5.9 Cooking Loss, Drip Loss, and Shear Force

Water holding capacity measures, and instrumental tenderness can be found in Table 3.8. No significant effects of genotype or diet were found on cooking loss. DS pigs (4.5%) were found to have significantly more 24 hour drip loss (P=0.037) than LB pigs (3.4%), indicating lower water holding capacity. LB pigs had lower shear force values than DS pigs (2.87 vs 3.31 kg, P=0.0012).

There were no significant effects of diet on shear force (P=0.680). There were no significant effects of diet on water holding capacity measurements, purge loss, drip loss, or cooking loss (P>0.194).

3.6 Discussion

The objective of this study was to characterize the effect of breed and diet on growth performance and carcass characteristics of LB and DS pigs. The DS pigs were chosen to represent modern-day genetics for pigs common in U.S. commercial production. The LB represented a

heritage breed of pig who has had little to no genetic selection for growth or carcass traits. The two diets selected, one composed of corn soybean meal and one supplemented with alfalfa and wheat middlings, were representative of diets commonly used in intensively managed and pasture-raised swine operations, respectively. The LB pigs were less metabolically efficient compared to the DS pigs. This can be seen by the fact that the LB pigs grew at slower rates, reaching slaughter age at significantly lower body weights compared to DS pigs. Additionally, LB pigs had similar feed intakes to that of DS pigs, yet LB pigs consumed more feed per kilogram of body weight resulting in poor feed efficiency. This is similar to findings from research in other heritage breeds like the Ossabaw pig (Wangsness et al., 1980) where it was reported that the pigs consumed less feed and lower rate of body weight gain, indicative of poor feed efficiency. Therefore, the LB pigs were less feed efficient compared to the DS pigs.

In addition to body weight, the LB pigs deposited muscle and fat differently compared to the DS pigs. The LB pigs had significantly smaller loin muscle areas and greater backfat compared the DS pigs regardless of diet, indicative of less lean growth in the LB pigs. This has been shown to be true for LB pigs and other minor swine breeds, such as the Meishan pig, when compared to domesticated swine breeds (White et al., 1995, Fahmey and Bernard, 1971; Whitley et al., 2012b). It is known that fat deposition varies among breeds and genders related to energy intake and use (Dafaer and Strathe, 2011). The LB pigs had increased fat accretion as determined in the live ultrasound scans where the LB pigs had 2.13 cm more tenth rib backfat and 15.9 cm^2 smaller loin muscle areas than DS pigs. These correlated to 2.1 cm more 10^{th} rib back fat and 28.6 cm^2 smaller loin muscle areas than DS pigs on carcass measurements. This is in agreement of other minor swine breeds to contain greater backfat deposits and reduced loin muscle areas (Suzuki et al., 2003; White et al., 1995). Heavier body weight pigs typically have greater backfat measures (Latorre et al., 2004; Ellis et al., 1996), however the LB had reduced live weights but greater backfat similar to past research on crossbred LB pigs (Whitley et al., 2012b; Fahmey and Bernard, 1971), and other minor breeds (Wood et al., 2004). Body fat deposition typically increases with weight (Correa et al., 2006), in the present study LB had accelerated backfat growth based on live scans compared to DS pigs indicating that genetics is the driving factor for the variation in fat deposition.

In regards to the wholesale cuts, the DS pigs yielded greater cut weights than LB pigs. This is likely due to genetic selection for DS pigs to accrete lean muscle and not fat (Schwab et al., 2006), resulting in a heavier pig at slaughter having greater yields than a light weight pig (Correa

et al, 2006). The LB was smaller in size than the DS pigs, which was a factor in the whole sale cut differences.

The LB pigs had a significant high final pH by 0.03 which could be indicative of a slightly lower glycolytic potential than the DS pigs, which is consistent with research in Meishan, Hampshire, and Pietrain breeds of pigs (Muller et al., 2002; Monin and Sellier, 1985). However, pre-slaughter handling has been found to impact post-mortem pH (de Oliveira et al., 2018) so it could be possible that the LB pigs were slightly less stressed just prior to slaughter. Stress indicators were not measured in this study, however, this breed is known for being docile and having a relaxed demeanor (Wallace, 1913; Livestock Conservancy, 2020). Additionally, the LB has extremely large ears which impairs their vision. It is unclear how this would positively or negatively impact their ability to adapt to transportation stress and a new environment at the slaughter house.

The current study utilized two methods to evaluate pork quality, the visual assessment using the NPPC scoring system and the objective Minolta digital color scores. The DS pigs scored within ideal commercial standards of pork quality according to the NPPC scoring system, however loin color was slightly below the ideal NPPC color score of 3.0 to 4.0, recording a mean of 2.92. Using subjective NPPC visual assessment scoring, the LB pigs had increased marbling and firmness scores, indicating increased intramuscular fat deposition, despite smaller loin muscle areas. Interestingly, the objective assessment Minolta colorimeter, did not detect genotypic differences in L*, a*, or b* measures. In the current study, the objective assessment found no genotypic difference, which may indicate that a consumer may perceive a darker color in LB pork although the overall score is within the ideal score set by the NPPC. The visual assessment may be less accurate than the subjective Minolta L* values observed in the present study without human measurement error. After cooking, the LB pork chops were more tender than the DS chops, which could be attributed to a possible difference in muscle fiber diameter. As this was not measured in this study, we can only speculate the LB pigs have a different muscle fiber diameter than that of the DS pigs as seen in past Berkshire research (Crawford et al., 2010). Proximate analysis would further determine how different the LB is from the DS pigs. Although the LB pigs were less lean than the DS pigs, the LB pigs scored slightly higher IMF content by visual assessment. Leanness is correlated with less intramuscular fat (Wood et al., 1996), in which the current study agrees that

the LB pigs visual scores were slightly higher than the lean DS pigs according to the NPPC standards.

Prediction of body weight from heart girths using the Groesbeck equation was found to be inaccurate by 13% or approximately 30 lbs of body weight. The equation was developed for 4 H pigs using major swine breeds to predict body weight within ±10lbs (Groesbeck et al., 2002). Show pigs have been selected for increased muscle mass and lean growth similar to commercial breeds, depositing very little subcutaneous fat. As muscle weighs more than fat, it is likely that the equation would need to be adjusted for use in heritage breeds which have reduced lean muscle body composition.

Due to the difference in body weights among the breeds, it is more accurate to evaluate organ weight differences as a percentage of body weight. At slaughter, the individual organ weights were not different among FIB and CON pigs, however, relative to liveweight the FIB pigs had greater organ masses than the CON evident in the whole visceral and liver percentages of live weight. This is in agreement with other research showing that diet can affect growth and development of animals (Kass et al., 1980), and increase lean growth (Schinckel et al., 2015; Pond et al., 1981). Pigs fed FIB developed larger gastrointestinal tracts to consume fibrous materials and accreted less fat. High fiber diets reduce digestibility and feed efficiency (Kass et al., 1980; Schinckel et al., 2015). In the current study, the pigs fed FIB had reduced ADG and G:F, but indicating that the pigs fed FIB grew slower, gained less weight per feed consumed. This is in agreement with past research with pastured free range swine (Kelly et al., 2007; Sather et al., 1997) and alfalfa supplemented diets (Kass et al., 1980; Pond et al., 1981) where a forage-based diet reduced growth and development with inefficient digestibility of feedstuffs. The inclusion of alfalfa meal in the diet has been previously studied (Pond et al., 1981; Powley et al., 1981), however did not interact with genotype, but did increase carcass leanness in the present study. Some minor swine breeds are thought to utilize dietary fiber more efficiently than major swine breeds (Lindberg, 2014; Len et al., 2009). This may be attributed to the ability to endure varied feed availability and environmental extremes (Edwards, 2005) as most heritage breeds are pasture-raised. It is difficult to compare outdoor production studies to determine differences in growth rates due to the wide variation in uncontrolled environment settings (Juska et al., 2013; Hoffman et al., 2003; Honeyman and Harmon, 2003). It has been found that short-term feeding of high fiber alters intestinal morphology (Jin et al., 1994) in order to adapt to the digestion of high fiber diets,

which is supported in this study where the pigs that consumed FIB had relatively larger organs to body weights (Anugwa et al., 1989; Len et al., 2009) than the CON pigs.

As discussed by Talbott et al. (2003) niche pork producers may potentially benefit from alternative diets, however a high fiber diet may be detrimental towards certain growth and carcass qualities in minor breeds. Minor breeds are fed various diets (Almeida et al., 2019), which considering the slow growth of minor breeds like the Large Black, Meishan, and British Saddleback (White et al., 1995; Kelly et al., 2007), it may be more advantageous to feed minor breeds to decrease the fat accretion (i.e. backfat). Minor breed genotypes traditionally have greater backfat measurements, firmness and darker meat color (Edwards, 2005; Wood et al., 2004), which is supported in the present study of the LB. Minor breeds are typically found in extensive systems, which in comparison to today's lean pork standards may be a suitable production environment for the LB to reach marketable weights (Whitley et al., 2012a; Lebret, 2008). As discussed by Edwards (2005), fat genotypes are more likely to experience metabolic stress due to poor diet quality and environment, though are potentially more fit to survive extreme environments. In the current study the backfat of the LB pigs fed FIB were still greater than that of DS pigs fed CON, further indicating that the effects of genotype were greater than that of dietary treatments.

In the current study, barrows for both breed types were heavier, and contained more backfat fat than gilts. This is in agreement with literature that suggests that boars are leaner than gilts (Latorre et al., 2003), however barrows gain fat more than gilts due to the absence of testosterone, an anabolic steroid. Estrogens increase fat synthesis in gilts, however with the absence of testosterone in the barrow, it accretes more fat than muscling (Daenfar and Strathe, 2011), in which the LB and DS sex differences were similar to that of major swine breeds.

3.7 Conclusion

Purebred Large Black pigs are slow growing and deposit more fat compared to Duroc-sired pigs, regardless of the type of diet fed to the animals. The LB pigs were determined to have satisfactory pork quality attributes, yet yielded nearly twice the backfat and reduced loin muscle areas compared to today's commercial crossbred pigs. The LB breed lacks lean growth, evident in the reduced percent lean and increased backfat depositions. Though a fat type pig breed, the LB was determined to have satisfactory meat quality attributes observed drip loss and shear force values. Further research should evaluate methods to mitigate fat deposition in the LB pig, such as

limit feeding practices, or the utilization of ractopamine to increase the lean growth of the LB pig which could possibly increase the productivity of this minor swine breed. Determination of the LB pigs genotypic protein deposition (Pd) could improve ideal Lysine and energy requirements for efficient protein deposition. LB breeders should ensure that diets are properly balanced to maximize animal growth, despite the fact that the LB pig has rapid fat deposition.

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Table 3.1. Composition of Control and Fiber diets (as fed basis)

	Phase 1[1]		Phase 2		Phase 3		Phase 4		Phase 5		Phase 6	
Ingredient. %	Control	Fiber	Control	Fiber	Control	Fiber	Control	Fiber	Control	Fiber	Control	Fiber
Corn	66.51	59.81	69.96	60.20	74.28	60.59	78.63	61.11	81.79	60.36	83.74	57.23
SBM, 47% CP	19.10	17.55	15.60	13.71	11.48	9.15	7.40	4.50	4.55	1.20	2.55	0.00
DDGS,7% fat	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Dehy. Alfalfa Meal	0.00	7.50	0.00	10.00	0.00	12.50	0.00	15.00	0.00	17.50	0.00	20.00
Wheat Midds	0.00	1.00	0.00	2.00	0.00	4.00	0.00	6.00	0.00	8.00	0.00	10.00
Swine Grease	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	1.37	1.09	1.30	0.93	1.25	0.80	1.22	0.69	1.14	0.53	1.12	0.43
MonoCal Phos.	0.53	0.53	0.47	0.45	0.37	0.30	0.21	0.10	0.16	0.00	0.23	0.00
Vit. Premix[2,3,4,5,6]	0.15	0.15	0.14	0.14	0.13	0.13	0.12	0.12	0.10	0.10	0.10	0.10
TM Premix [7,8,9,10,11]	0.10	0.10	0.09	0.09	0.08	0.08	0.07	0.07	0.05	0.05	0.05	0.05
Se Premix[12]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.03	0.03	0.03	0.03
Phytase[13]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.30	0.30	0.25	0.25	0.25	0.25
Lysine-HCL	0.42	0.44	0.40	0.42	0.40	0.42	0.40	0.42	0.36	0.38	0.36	0.34
DL-Methionine	0.09	0.10	0.06	0.08	0.05	0.07	0.02	0.05	0.00	0.03	0.00	0.02
L-Threonine	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.12	0.09	0.10	0.09	0.08
L-Tryptophan	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.03	0.03	0.02
Clarify[14]	0.07	0.07	0.09	0.09	0.07	0.07	0.08	0.08	0.09	0.09	0.10	0.10
Defusion[15]	0.00	0.00	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Table 3.1. continued

Calculated Nutrients												
ME, Kcal/kg	3329.5	3206.2	3324.9	3161.4	3338.7	3124.7	3351.0	3089.6	3361.0	3052.2	3360.8	3003.6
NE, Kcal/kg	2498.3	2375.1	2512.4	2346.5	2544.1	2325.4	2574.4	2306.2	2596.7	2278.5	2506.7	2231.3
ADF, %	4.14	6.19	4.06	6.82	3.97	7.46	3.88	8.11	3.82	8.79	3.77	9.51
NDF, %	10.68	13.38	10.70	14.48	10.76	15.87	10.82	17.26	10.88	18.66	10.89	20.00
CP, %	17.79	17.96	16.35	16.70	14.75	15.29	13.14	13.85	11.98	12.89	11.18	12.73
SID Lysine, %	1.00	1.00	0.90	0.90	0.80	0.80	0.70	0.70	0.60	0.60	0.55	0.55
Ca, %	0.70	0.70	0.65	0.65	0.60	0.60	0.55	0.55	0.50	0.50	0.50	0.50
P, %	0.50	0.50	0.47	0.48	0.43	0.44	0.38	0.39	0.36	0.37	0.36	0.38
Avail. P, %	0.32	0.32	0.30	0.30	0.27	0.27	0.23	0.23	0.22	0.22	0.22	0.22

[1]Phase 1 was fed from d 63 to 84 of age; Phase 2 was fed from d 84 to 105 of age; Phase 3 was fed from d 105 to 126 of age; Phase 4 was fed from d 126 to 147 of age; Phase 5 was fed from d 147 to 168 of age; Phase 6 was fed from d 168 of age to marketing

[2]Provided per kilogram of the diet (0.15% inclusion): vitamin A, 3,969 IU; vitamin D3, 397 IU; vitamin E, 26.5 IU; vitamin K, 1.3 mg; riboflavin, 5.3 mg; pantothenic acid, 13.2 mg; niacin, 19.8 mg; B12, 23.2 mg

[3]Provided per kilogram of the diet (0.14% inclusion): vitamin A, 3,704 IU; vitamin D3, 370 IU; vitamin E, 24.7 IU; vitamin K, 1.2 mg; riboflavin, 4.9 mg; pantothenic acid, 12.3 mg; niacin, 18.5 mg; B12, 21.6 mg

[4]Provided per kilogram of the diet (0.13% inclusion): vitamin A, 3,340 IU; vitamin D3, 344 IU; vitamin E, 22.9 IU; vitamin K, 1.1 mg; riboflavin, 4.6 mg; pantothenic acid, 11.5 mg; niacin, 17.2 mg; B12, 20.0 mg

[5]Provided per kilogram of the diet (0.12% inclusion): vitamin A, 3,175 IU; vitamin D3, 318 IU; vitamin E, 21.2 IU; vitamin K, 1.1 mg; riboflavin, 4.2 mg; pantothenic acid, 10.6 mg; niacin, 15.9 mg; B12, 18.5 mg

[6]Provided per kilogram of the diet (0.10% inclusion): vitamin A, 2,646 IU; vitamin D3, 265 IU; vitamin E, 17.6 IU; vitamin K, 0.9 mg; riboflavin, 3.5 mg; pantothenic acid, 8.8 mg; niacin, 13.2 mg; B12, 15.4 mg

[7]Provided per available minerals kilogram of the diet (0.10% inclusion): iron, 97 mg; zinc, 97 mg; manganese, 12.0 mg; copper, 9.0 mg; iodine, 0.37 mg

[8]Provided per available minerals kilogram of the diet (0.09% inclusion): iron, 87 mg; zinc, 87 mg; manganese, 10.8 mg; copper, 8.1 mg; iodine, 0.33 mg

[9]Provided per available minerals kilogram of the diet (0.08% inclusion): iron, 78 mg; zinc, 78 mg; manganese, 9.6 mg; copper, 7.2 mg; iodine, 0.29 mg

[10]Provided per available minerals kilogram of the diet (0.07% inclusion): iron, 68 mg; zinc, 68 mg; manganese, 8.4 mg; copper, 6.3 mg; iodine, 0.26 mg

[11]Provided per available minerals kilogram of the diet (0.05% inclusion): iron, 48.5 mg; zinc, 48.5 mg; manganese, 6.0 mg; copper, 4.5 mg; iodine, 0.18 mg

[12]Provided 0.3 ppm Se (0.05% inclusion) or 0.15 ppm Se (0.025% inclusion).

[13]Provided 600 FTU of phytase per kg of the diet (Phyzyme, Danisco Animal Nutrition/DuPont, St. Louis, MO)

[14]Clarifly (Central Life Sciences, Schaumburg, IL) provided 6.7, 6.0, 5.4, and 4.7 ppm diflubenzuron as a larvicide in the diet when included at 0.10, 0.09, 0.08, and 0.07%.

[15]Defusion (Provimi, Brookville, OH) a blend of feed preservatives and other ingredients.

Table 3.2. Effect of diet and genotype on growth performance

Genetics	DS		LB			Statistical Significance, *P*<			
Diet	CON	FIB	CON	FIB	SEM	Genetics	Diet	G x D	Rep
Phase 1, d 0-21[1]									
d 0 BW, kg	16.7	16.7	16.8	16.8	0.69	0.846	0.995	0.995	-
ADG, kg/d	0.70	0.63	0.47	0.50	0.03	0.012	0.745	0.227	-
ADFI, kg/d	1.33	1.29	1.18	1.35	0.06	0.494	0.340	0.166	-
G:F	0.52	0.49	0.40	0.38	0.01	0.001	0.060	0.714	-
d 21 BW, kg	32.7	31.2	28.2	28.4	1.32	0.070	0.675	0.591	-
Phase 2, d 21-42									
Phase 2 Initial BW, kg	34.2	33.0	31.2	32.1	0.84	0.040	0.886	0.264	<0.001
ADG, kg/d	0.89	0.89	0.53	0.61	0.05	<0.001	0.486	0.477	0.482
ADFI, kg/d	2.09	1.97	1.84	1.93	0.12	0.240	0.892	0.390	0.038
G:F	0.43	0.45	0.30	0.32	0.03	0.001	0.553	0.990	0.287
Phase 3, d 42-63									
Phase 3 Initial BW, kg	51.5	50.5	42.6	44.2	0.96	<0.001	0.784	0.196	0.001
ADG, kg/d	0.84	0.81	0.70	0.61	0.03	<0.001	0.075	0.362	<0.001
ADFI, kg/d	2.25	2.26	2.21	2.38	0.08	0.646	0.317	0.347	0.873
G:F	0.37	0.36	0.31	0.26	0.01	<0.001	0.020	0.092	<0.001
Phase 4, d 63-84									
Phase 4 Initial BW, kg	69.1	67.5	57.2	57.0	0.97	<0.001	0.358	0.452	<0.001
ADG, kg/d	1.04	0.91	0.73	0.59	0.06	0.001	0.058	0.960	0.820
ADFI, kg/d	2.60	2.73	2.62	2.54	0.16	0.586	0.867	0.533	0.342
G:F	0.40	0.33	0.28	0.24	0.01	<0.001	0.004	0.468	0.275
Phase 5, d 84-105									
Phase 5 Initial BW, kg	90.9	86.5	73.3	69.5	1.83	<0.001	0.048	0.857	0.002
ADG, kg/d	0.88	0.77	0.69	0.59	0.05	0.006	0.077	0.950	0.400
ADFI, kg/d	3.07	3.11	2.84	2.85	0.15	0.135	0.885	0.901	0.239
G:F	0.29	0.25	0.25	0.21	0.02	0.037	0.046	0.999	0.989
Phase 6, d 105-Market									
Phase 6 Initial BW, kg	109.9	103.1	88.3	82.2	2.14	<0.001	0.014	0.877	0.005
ADG, kg/d	0.78	0.75	0.72	0.59	0.06	0.112	0.204	0.405	0.207
ADFI, kg/d	2.88	2.96	2.59	2.86	0.15	0.218	0.260	0.526	0.612

Table 3.2 continued

G:F	0.27	0.26	0.28	0.20	0.02	0.279	0.035	0.129	0.285
Overall, d 21-Market									
ADG, kg/d	0.92	0.85	0.72	0.62	0.03	<0.001	0.020	0.534	0.002
ADFI, kg/d	2.62	2.65	2.46	2.56	0.11	0.299	0.579	0.778	0.213
G:F	0.35	0.32	0.29	0.24	0.01	<0.001	0.007	0.294	0.020
Market BW, kg	130.3	122.8	106.4	96.6	3.80	<0.001	0.045	0.768	0.995

[1]Phase 1 includes replicate 2 only.

Table 3.3. Effect of diet and genotype on livescan ultrasound

Genetics	DS		LB			Statistical Significance, $P <$				
Diet	CON	FIB	CON	FIB	SEM	Genetics	Diet	G x D	Sex	Rep
d 42 Live Weight, kg	51.0	52.1	42.5	44.0	2.18	<0.001	0.543	0.933	0.842	0.026
d 84 Live Weight, kg	90.5	88.7	73.2	70.7	2.70	<0.001	0.425	0.902	0.497	0.004
Market Live Weight, kg	131.5	122.9	106.6	97.7	4.01	<0.001	0.036	0.967	0.673	0.702
d 42 Last Rib BF, cm	0.80	0.80	1.61	1.56	0.08	<0.001	0.765	0.765	1.00	<0.001
d 84 Last Rib BF, cm	1.10	1.14	1.99	1.75	0.10	<0.001	0.324	0.180	0.980	0.180
Market Last Rib BF, cm	1.95	1.85	4.25	3.77	0.22	<0.001	0.197	0.393	0.010	0.037
d 42 10th Rib BF, cm	0.89	0.88	1.71	1.59	0.11	<0.001	0.533	0.610	0.533	0.164
d 84 10th Rib BF, cm	1.09	1.08	2.16	2.04	0.11	<0.001	0.531	0.607	0.032	0.395
Market 10th Rib BF[1], cm	1.85	1.93	4.38	3.38	0.23	<0.001	0.056	0.028	0.004	0.099
d 42 Loin Depth, cm	3.01	2.75	2.04	2.20	0.15	<0.001	0.737	0.161	0.503	0.053
d 84 Loin Depth, cm	4.03	3.86	3.09	3.22	0.17	<0.001	0.924	0.380	0.163	0.042
Market Loin Depth, cm	6.30	6.18	3.99	3.77	0.24	<0.001	0.462	0.840	0.766	0.139
d 42 LEA, cm^2	15.4	15.0	9.1	9.4	0.94	<0.001	0.962	0.688	0.558	<0.001
d 84 LEA, cm^2	25.9	22.2	14.6	15.9	1.35	<0.001	0.362	0.071	0.693	0.521
Market LEA, cm^2	47.7	46.4	31.8	30.5	2.32	<0.001	0.548	0.995	0.834	<0.001
Market FFL, kg	48.5	46.4	28.1	28.0	1.31	<0.001	0.405	0.453	0.079	0.451
Market Percent Lean[2], %	49.8	51.1	36.0	39.2	1.60	<0.001	0.168	0.543	0.036	0.267

[1]Market 10th Rib BF G x S interaction (P<0.034).

[2]Percent lean calculated from 10th rib ultrasound scans.

Table 3.4. Effect of diet and genotype on dissected organ mass

Genetics	DS		LB			Statistical Significance, *P* <							
Diet	CON	FIB	CON	FIB	SEM	Genetics	Diet	G x D	Sex	G x S	D x S	G x D x S	Rep
Live Weight, kg	130.3	124.2	106.2	95.6	2.69	<0.001	0.002	0.388	0.298	0.456	0.980	0.241	0.567
EBW[1], kg	116.9	110.0	95.1	84.6	2.54	<0.001	0.001	0.441	0.257	0.359	0.798	0.318	0.747
Visceral, kg	13.5	14.1	11.0	11.0	0.40	<0.001	0.430	0.350	0.835	0.408	0.149	0.117	0.074
Visceral LW, %	10.3	11.4	10.4	11.5	0.37	0.809	0.003	0.973	0.559	0.156	0.197	0.699	0.242
Visceral CW[2], %	13.4	15.3	14.1	16.1	0.58	0.160	0.001	0.903	0.666	0.146	0.210	0.721	0.393
Leaf fat, kg	1.70	1.55	2.37	1.82	0.14	0.001	0.015	0.161	0.001	0.417	0.686	0.037	0.043
Leaf fat CW, %	1.68	1.65	3.00	2.64	0.14	<0.001	0.142	0.228	0.001	0.628	0.685	0.035	0.010
Kidneys, kg	0.37	0.37	0.27	0.30	0.01	<0.001	0.125	0.281	0.272	0.606	0.004	0.912	0.011
Liver, kg	1.69	1.73	1.37	1.36	0.04	<0.001	0.756	0.613	0.992	0.321	0.207	0.211	0.004
Liver LW, %	1.31	1.45	1.28	1.40	0.03	0.175	<0.001	0.751	0.167	0.141	0.273	0.607	0.025
Liver CW, %	1.70	1.93	1.73	1.93	0.04	0.671	<0.001	0.702	0.087	0.082	0.212	0.812	0.012
Intestine, kg	4.47	4.18	3.89	3.53	0.23	0.023	0.189	0.892	0.841	0.892	0.700	0.500	0.349
Intestine LW, %	3.35	3.33	3.45	3.59	0.17	0.318	0.744	0.628	0.651	0.849	0.456	0.882	0.596
Intestine CW, %	4.38	4.46	4.65	4.97	0.25	0.148	0.452	0.623	0.511	0.865	0.535	0.818	0.548
Total GI[3], kg	6.63	5.99	5.84	5.20	0.26	0.011	0.033	0.989	0.179	0.875	0.495	0.659	0.279
Total GI LW, %	4.98	4.77	5.18	5.29	0.21	0.115	0.830	0.474	0.480	0.990	0.609	0.579	0.081
Total GI CW, %	4.82	5.18	5.14	4.81	0.33	0.944	0.962	0.314	0.625	0.656	0.520	0.554	0.031
Sexual Maturity[4], %	100% (6/6)	74% (5/7)	0% (0/6)	25% (2/8)	13.0	0.021	0.936	-	-	-	-	-	-

[1]Empty body weight calculated as viscera subtracted from live weight.
[2]Hot carcass weight.
[3]Total gastrointestinal tract includes spleen and mesentery.
[4]Binary variable tested for genetics and dietary treatment effects only.

Table 3.5. Effect of diet and genotype on carcass characteristics

Genetics	DS		LB			Statistical Significance, *P* <							
Diet	CON	FIB	CON	FIB	SEM	Genetics	Diet	G x D	Sex	G x S	D x S	G x D x S	Rep
HCW, kg	100.4	92.7	78.3	68.5	2.18	<0.001	<0.001	0.586	0.223	0.271	0.813	0.367	0.529
CCW, kg	98.6	91.3	76.8	67.6	2.15	<0.001	<0.001	0.586	0.223	0.271	0.813	0.367	0.529
Carcass Length, cm	82.9	83.4	78.4	77.3	0.82	<0.001	0.557	0.249	0.187	0.165	0.826	0.367	0.200
Dressing Percentage, %	75.9	73.7	73.4	71.3	0.44	<0.001	<0.001	0.933	0.739	0.524	0.160	0.846	0.007
First Rib, cm	4.48	3.81	5.38	4.73	0.21	<0.001	0.001	0.991	0.006	0.940	0.986	0.488	0.387
Tenth Rib, cm	2.44	2.25	4.77	4.05	0.18	<0.001	0.014	0.140	<0.001	0.033	0.718	0.063	0.023
Last Rib, cm	3.01	2.75	4.51	3.69	0.24	<0.001	0.022	0.235	0.006	0.444	0.792	0.116	0.003
Last Lumbar, cm	2.89	2.66	4.32	3.74	0.16	<0.001	0.011	0.247	0.047	0.787	0.413	0.604	0.177
Loin Depth, cm	6.87	6.67	3.78	3.80	0.12	<0.001	0.326	0.296	0.134	0.012	0.640	0.678	0.027
LEA, cm	52.59	52.17	23.87	23.64	1.42	<0.001	0.769	0.951	0.047	0.155	0.762	0.556	0.449
FFL, kg	53.5	50.7	28.1	26.3	1.07	<0.001	0.012	0.632	0.012	0.005	0.576	0.537	0.085
Percent Lean[1], %	53.5	54.9	36.1	38.5	0.81	<0.001	0.018	0.550	<0.001	0.004	0.569	0.067	0.030

[1]Ribbed carcass percent lean =[(7.2+(HCWx0.44)+(LEAx3.88)-(10th rib fat depthx18.75))/HCW]x100

Table 3.6. Effect of diet and genotype on wholesale cuts

Genetics	DS		LB			Statistical Significance, *P* <							
Diet	CON	FIB	CON	FIB	SEM	Genetics	Diet	G x D	Sex	G x S	D x S	G x D x S	Rep
Boston Butt, kg	6.69	6.30	5.03	4.58	0.22	<0.001	0.023	0.856	0.242	0.517	0.258	0.358	0.006
Picnic, kg	4.42	4.20	3.29	2.97	0.14	<0.001	0.039	0.698	0.277	0.234	0.360	0.979	0.003
Jowl, kg	1.32	1.30	1.46	1.08	0.06	0.447	0.001	0.002	0.005	0.788	0.959	0.318	<0.001
Neckbones, kg	1.13	1.05	0.89	0.78	0.07	<0.001	0.130	0.810	0.049	0.982	0.848	0.926	<0.001
Loin, kg	12.40	10.93	10.04	8.17	0.35	<0.001	<0.001	0.536	0.032	0.870	0.922	0.057	0.765
Spareribs, kg	1.54	1.44	1.20	1.03	0.04	<0.001	0.002	0.402	0.031	0.151	0.158	0.811	0.001
Belly, kg	7.64	6.97	6.88	5.93	0.26	<0.001	0.001	0.526	0.566	0.845	0.799	0.466	0.689
Ham, kg	11.55	11.31	8.19	7.59	0.29	<0.001	0.116	0.496	0.907	0.185	0.877	0.447	0.001

Table 3.7. Effect of diet and genotype on NPPC pork quality

Genetics	DS		LB			Statistical Significance, *P* <							
Diet	CON	FIB	CON	FIB	SEM	Genetics	Diet	G x D	Sex	G x S	D x S	G x D x S	Rep
Firmness	2.71	2.68	3.55	3.42	0.12	<0.001	0.437	0.639	0.319	0.586	0.703	0.089	0.595
Color	2.91	2.94	3.36	3.22	0.11	0.002	0.563	0.435	0.679	0.145	0.207	0.312	0.176
Marbling	2.54	2.54	3.08	3.19	0.14	<0.001	0.691	0.714	0.498	0.283	0.097	0.027	<0.001

Table 3.8. Effect of diet and genotype on pH, instrumental color and tenderness

Genetics	DS		LB			Statistical Significance, *P* <							
Diet	CON	FIB	CON	FIB	SEM	Genetics	Diet	G x D	Sex	G x S	D x S	G x D x S	Rep
24 h pH	5.60	5.61	5.63	5.64	0.01	0.012	0.452	0.803	0.974	0.488	0.708	0.215	0.007
Minolta L*	48.50	47.38	48.64	47.66	0.72	0.724	0.144	0.898	0.968	0.699	0.061	0.155	0.607
Minolta a*	8.77	7.84	7.94	7.98	0.45	0.408	0.274	0.268	0.178	0.958	0.307	0.473	0.122
Minolta b*	6.57	5.86	5.72	5.70	0.31	0.091	0.210	0.252	0.168	0.672	0.065	0.941	<0.001
Drip Loss, %	4.57	4.49	3.41	3.23	0.46	0.009	0.821	0.920	0.512	0.366	0.971	0.032	0.123
Purge Loss, %	5.00	6.18	8.88	8.83	0.69	<0.001	0.386	0.351	0.957	0.447	0.019	0.004	<0.001
Cooking loss, %	20.8	23.6	20.8	20.7	1.05	0.157	0.194	0.149	0.654	0.413	0.288	0.056	0.131
Shear Force[1], kg	3.33	3.32	2.80	2.92	0.14	<0.001	0.680	0.614	0.243	0.235	0.729	0.691	0.693

[1]Aged 7 days.

CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTION

4.1 Conclusions

The overreaching objective of this project was to understand the physiology of one minor swine breed, the Large Black, and its growth and development during the life phases of pork production in order to facilitate a genetic import for this breed. Finding ways to improve upon subsequent reproductive management practices in a breed with limited breeding stock is an achievement that was realized with the creation of the piglets from imported genetics. As other minor breeds are also considered endangered, there is a need to continuously find ways to conserve these rare breeds before the genetics are lost. In 2004, it was estimated that 300 livestock breeds were lost to extinction over the course of 15 years (Cardellino, 2004), however that number has likely increased, with some estimating at least one rare breed lost every few days. Chapter 2 demonstrates that a frozen semen artificial insemination breeding protocol can be implemented in the Large Black sow to utilize frozen boar semen. Chapter 3 explores the role that genetics and diet plays in the growth and development of the Large Black compared to commercial crossbred pigs managed under commercial conditions.

Chapter 2 describes the characteristics of the donated Large Black pigs, and subsequent reproductive performance. Under intensively managed conditions, Large Black gilts that were able to conceive with frozen American Large Black semen, were selected and able to achieve a 50% conception rate with less than 2×10^9 total motile sperm cells. The sows that were able to conceive in replicates 1 and 2, were likely more fertile as they managed to conceive in later replicates as well. Furthermore, it was demonstrated that the Large Black sow is able to conceive when her reproduction is controlled through the utilization of estrous synchronization and ovulation induction. Though this small study is confounded by multiple factors, it is still a success as piglets were created from imported semen. The ability to achieve a 50% conception rate with 0.34×10^9 motile sperm cells from frozen semen has further implications beyond the minor swine breeds. Spencer et al. (2010) laid the groundwork that 1×10^9 total motile sperm cells is acceptable to achieve pregnancy using frozen semen, especially in conservation situations. However, in this study, it became evident that a known successful reproductive history may increase reproductive success when using frozen semen where the quantity of semen is limited. When considering breed

conservation, it is typical that semen quantity is limited and, therefore, these findings that less than 1×10^9 total motile sperm cells from frozen semen can establish pregnancy is exciting. Progressive low input swine breeders utilizing estrous synchronization, ovulation induction, and frozen boar semen are likely to benefit from incorporating ART in individual herds providing avenues for minor breed improvement and preservation. One downfall to this project is that the piglets were not created on the farms that typically breed the Large Black pigs. Whether small breeders could manage their animals in order to have similar success remains unknown.

Chapter 3 characterizes the growth performance of the Large Black in comparison to commercial crossbred pigs when fed with or without a diet of fibrous dehydrated alfalfa materials. The Large Black pig has had virtually no genetic selection pressure for lean growth or carcass traits. It has been discussed as early as 1913, that the Large Black accretes fat rapidly (Wallace, 1913), and found that by crossbreeding the Large Black to other swine breeds that it presents challenges of reduced lean (Fahmey and Bernard, 1971). Therefore, it is not surprising that the Large Black pig was shown to have reduced growth rates with lower lean and higher fat accretion. However, it was unknown what the effects of diet on growth and carcass traits were in this heritage breed. The alfalfa supplemented diet designed to mimic a pasture-raised pig diet, resulted in decreased growth rates and slight changes in carcass traits. None of these were as significant as the effects of genotype, however. It is important to note that the diets used in this study were designed to meet all of the nutritional requirements for the animals at their respective stages of growth. Oftentimes pasture-raised pigs may not be fed complete diets considering that niche markets are popular among short term breeders and, therefore, not grow at similar rates to the animals used in this study. The information provided by this study is beneficial to the small farms that are marketing this pork in niche production systems. This data will allow breeders to have proof that the meat products they are marketing are higher marbled.

This project utilized donated animals from various small farms across the U.S. The producers were happy to be involved and provide information about their farms, animals, and management practices. By surveying the producers, it became evident that the Large Black was attractive to niche pork producers with a wide variety of farm styles and management practices. All of the Large Black donors that were surveyed are dedicated breeders and highly passionate about the conservation of this rare breed. One of the biggest challenges to conservation of these rare breeds is finding breeders with the dedication to the breed to make the long-term commitment

to conservation. This requires coordination among the breeders across the country as well as managing pure-bred pedigree information and registries. Maiwashe and Blackburn (2004) discuss that the limiting factor in breed conservation is short-term breeders which results in negative impacts on conservation of minor breeds. Based on the donor surveys, 66% have raised Large Blacks for more than 5 years, suggesting that with the Large Black, breeders are invested in the long-term efforts to conserve the breed. These progressive producers that participated in this project are willing to take financial risk to conserve the breed they are passionate about. At the conclusion of this project, the piglets and the management of the imported genetics via live animals is handed back to the breeders. Other genetic imports in other species have resulted in animals born with from the imported semen. However, in most cases the presence of those genetics in the national herd is eliminated within 5 or so generations. Based on the commitment of the breeders that participated in this project, we are optimistic that this import will remain successful with coordinated management of the imported genetics.

Pork production, regardless of type of management system, is more intensive than other livestock industries. Rare livestock breeds are at greater risk of loss than commercial breeds, due to limited productivity (Roberts and Lamberson, 2015). In recent years, rare breed conservation has been dependent on several cooperative partners. Without mutual cooperation, it may be impossible to achieve the momentum that the American Large Black breed has accomplished following the 2017 reproductive failure. The 2019 Large Black Swine Workshop laid groundwork for cooperative efforts to continue. The role of the University, a non-government organization, has been successful in achieving the primary objective of creating Large Black pigs and maintaining cooperative relationships. It is not known how the imported Large Black pig genetics will impact the American Large Black herd, however relationships among the breeders have been formed which may be beneficial in future management of the Large Black breed in America. In terms of genetic preservation, the role of Purdue University in minor breed conservation has been defined as an intermediate between the government, non-profit organizations, and the individual breeders, providing a gateway to information and improved cooperative relations. This has demonstrated a cooperative approach to preserving the Large Black, and serves as an example for future genetic preservation work. However, it is the responsibility of the invested Large Black breeders to continue ensuring cooperative conservation efforts, and maintain valuable cooperative

relationships with government, universities, and non-profit organizations. These relationships allow for further successful conservation opportunities.

4.2 Future Direction

Further research is warranted into the utilization of low total motile sperm counts from frozen-thawed semen when used with ovulation induction. Further exploration of timing of FTS inseminations based on ovulation induction when incorporating OvuGel® may provide an avenue for improved FTS fertility among major swine breeds. The Large Black breed is reliant on breed management decisions, and breeders should maintain cooperative efforts. Selection for lowly heritable, however beneficial reproductive traits may potentially assist with improving the productivity of the Large Black breed.

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APPENDIX A. DONOR SURVEYS

A.1.1 Donor Survey Questions

A.1.1.1 Section 1. General Farm Questions

1. How many years have you raised Large Black Hogs?
2. Do you raise any other swine besides Large Black Hogs, if so what breed?
3. Have you crossbred your Large Blacks? If so, with what breed?
4. What interested you to raise Large Black Hogs?
5. Is farming your only source of income? (Yes or No)
6. Do you have any Large Black show pigs? If so, what class(es) were they in?
7. What is the size of your Large Black breeding herd? (Select a range: 0-10, 10-20, 20-30, 30-40, >40)
8. Describe your farm's pasture system. (Open ended)
9. What health issues is common on your farm? (ex: louse, pneumonia)
10. What was the furthest distance you have traveled to pick up/ deliver breeding stock?

A.1.1.2 Section 2. Breeding Management Questions

1. What breeding considerations do you have when selecting a piglet out of a litter to be a breeder? Please list any and all:
2. Have you noticed certain Large Black bloodlines have different traits? If yes, please elaborate on each that you have (hair type, temperament, growth rates, etc.)?
3. Do you keep track of the day you turn the boar out with the females, to when farrowing happens? (Yes or No)
4. About how many boars do you keep on your farm? What are the current ages of those boars?
5. Do you bring in new boars? How old are they when you turn them out with your sows?
6. What is the age of the gilts when you first turn out with the boar?
7. What age do you castrate your males that are not selected to become a boar?
8. Have you ever used artificial insemination (Yes or No), what are your thoughts on it?

9. Would you be willing to learn artificial insemination, to breed the Large Blacks? (Yes, Unsure, or No)

A.1.1.3 Section 3. Farrowing Management Questions

1. What time of year do you typically farrow?
2. How many times of year do you farrow?
3. What do you farrow your pigs in (hut, open crate, etc.)? Please describe:
4. Do you attend any or all farrowings? (Yes or No). Have you provided assistance if farrowing trouble occurred?
5. What was last year's average litter size on your farm?
6. At what age do you wean your pigs? (Please select: 21 days, 42 days, varies because it depends on size of piglets, or please write how many days)
7. Do you practice fostering pigs? (Yes or No)
8. When do you turn the newly weaned sow back with the boar? Please select: "immediately after weaning", or "keep them separate for a period" (if you farrow multiple times a year)

A.1.1.4 Section 4. Products Questions

1. What do you feed your finishing hogs? How different is it from what you feed your breeding stock?
2. When do you slaughter? What age do you typically slaughter?
3. What is your live weight and carcass weights at slaughter?
4. Do you raise feeder pigs to sell? (Yes or No)
5. Who butchers your pigs?
6. What market do you sell your pork to? (freezer, farmer's, etc.)
7. How do you market your pork to your customers? (local, sustainable, etc.)
8. In your opinion, what is the best cut of meat from the Large Blacks?

A.1.1.5 Section 5. Large Black Project at Purdue Question

1. What do you hope to get out of the Purdue breeding project? (open ended)

A.1.2 Donor Survey Results

A total of 6 donors out of 9 responded to the Large Black survey, answering questions from all five topic sections. Farms located in Illinois, North Carolina, Pennsylvania, Indiana, Michigan, and Ohio participated in this donor survey providing a diverse profile of Large Black farmers spread throughout the United States during March through April 2019 time period. All questions were answered and provided diverse answers to each question. No question was answered as non-applicable or left unanswered by the 6 respondents. Answers were categorized to condense the responses received in this donor survey.

A.1.2.1 Section 1. General Farm Answers

Large Black donor characteristics can be found in Figure A.1 All surveyed donors listed farming as not their primary income. 66% of respondents do not raise any other swine breeds on their farms, however of the 33% that do, the raise Yorkshire, Berkshire, and Tamworth breeds. Those same farms that raise other swine breeds actively crossbreed their purebred Large Blacks to Yorkshire, Gloucestershire Old Spots, Berkshire, and Tamworth, most notably heritage pigs. 50% of respondents raise Large Blacks due to their conservation status as an endangered breed, with 33% raising Large Blacks due to their unique foraging ability and temperament that suit their farm type. One farm surveyed listed that they also raise their Large Blacks for county fair show pigs. As a unique story, this farm successfully petitioned for their county fair board to include an "All Other Breed" showing class, allowing their Large Black pigs to be included. All donors raise their Large Blacks in pasture settings, with all but one listing their pastures as rotational grazing systems. The donors were surveyed an open question about health issues on their farms, and 50% noted both external and internal parasites as major health issues. Two farms noted no known health issues on their farms. Due to the limited number of Large Blacks dispersed throughout the United States, the average distance travelled to transport breeding stock was 366.6 miles, with the furthest estimated to be 600 miles in one direction.

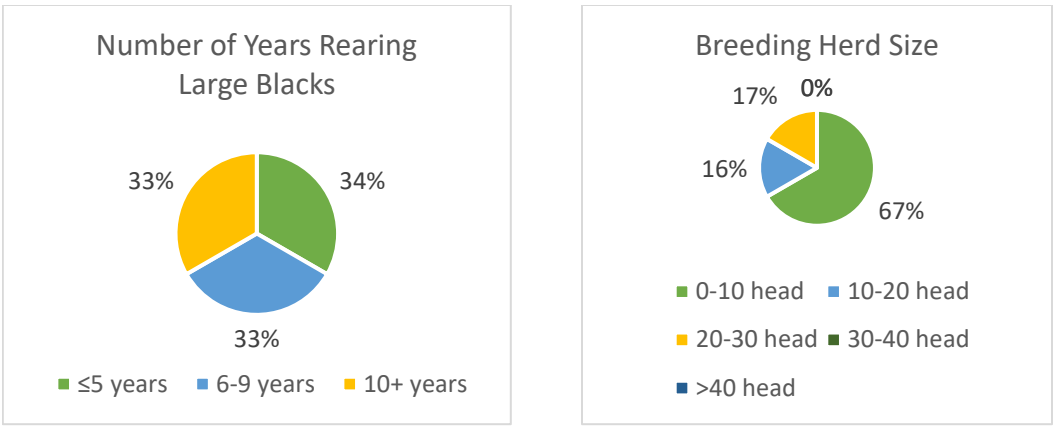

Figure A.1. This figure profiles the Large Black donor farm characteristics from survey respondents.

A.1.2.2 Section 2. Breeding Management Answers

All but one respondent maintain breeding records associated with the date the boar is turned out with the breeding sow herd; that respondent actively maintains a free range Large Black herd. All respondents actively maintain between 1 and 2 Large Black boars on site at their farms. The average age of those boars is 3.71 years of age, with the oldest being recorded as 7 years of age. When asked about age of the boar when turning out with the sows, most farms record at least 1 year of age with one farm leaving the boars out with sows all year long. All farms varied on the age of castration, with the oldest age being 8 weeks of age. 66% stated castration occurs less than 21 days of age.

All respondents responded to questions about artificial insemination, with 50% having experience with using artificial insemination at least one previous time. 2 respondents stated that AI was unnecessary, with 1 additional respondent stating that it is difficult to time from previous experience. In terms of implementing artificial insemination program in their own Large Black herd, 83% of respondents stated that they would be willing to learn artificial insemination to breed their Large Blacks. One respondent answered that they would be unsure to learn artificial insemination. 0% indicated that they would not be willing to learn artificial insemination.

A.1.2.3 Section 3. Farrowing Management Answers

83% of respondents farrow in at least one to two defined seasons per year. Of those respondents that farrow one to two times per year, farrowing times fall within spring (March to June) and fall (September to November) months. All provide shelters for farrowing, with 66% farrowing in a provided pen in barn. The 2018 average farm litter size was estimated to be 8.3, with respondents varying in litter sizes described along with weaning age in figure A.2.

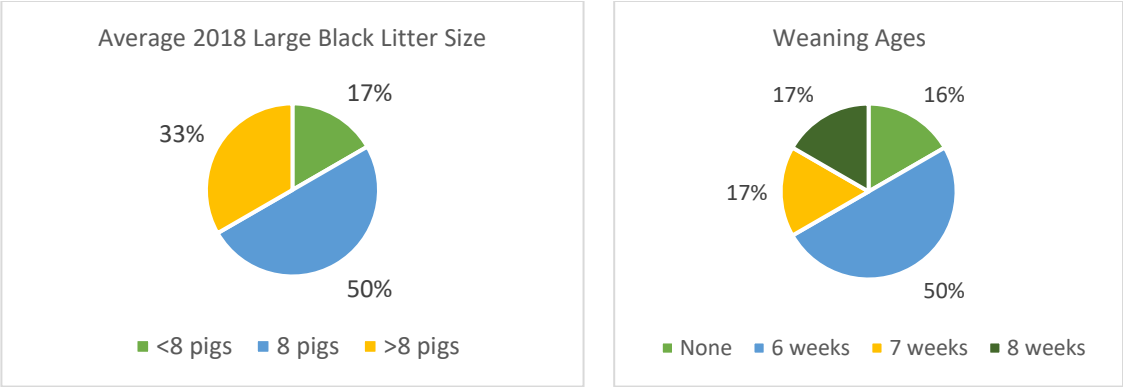

Figure A.2. This figure describes surveyed donor farrowing characteristics.

A.1.2.4 Section 4. Products Answers

All answers pertaining to product questions varied. Finishing pigs were fed items that ranged from “sweet feed” to “hay”. Estimated live weights varied and ranged between 220 to 350 lbs, with carcass weights ranging between 180 to 267 lbs. All respondents slaughter pigs at local processors. A wide variety of terms were associated with marketing, with terms ranging from “pasture raised” to “non-GMO”. All respondents market their pork products for freezer or farmer’s markets. Several respondents responded favoring processed products such as lard, and bacon.

A.1.2.5 Section 5. Large Black Project at Purdue Question

Finally, when asked about hopes about the Large Black project at Purdue, all respondents stated hopes for successful artificial insemination trial leading to new genetics for them to receive. One respondent stated that any information from a meat study would be beneficial for marketing

aspects. Another respondent stated that in addition to new genetics, they hope that the project encourages new breeders to raise more Large Black pigs.