

Decoding the western diet's impact on cognition and blood biomarkers in Ossabaw pigs

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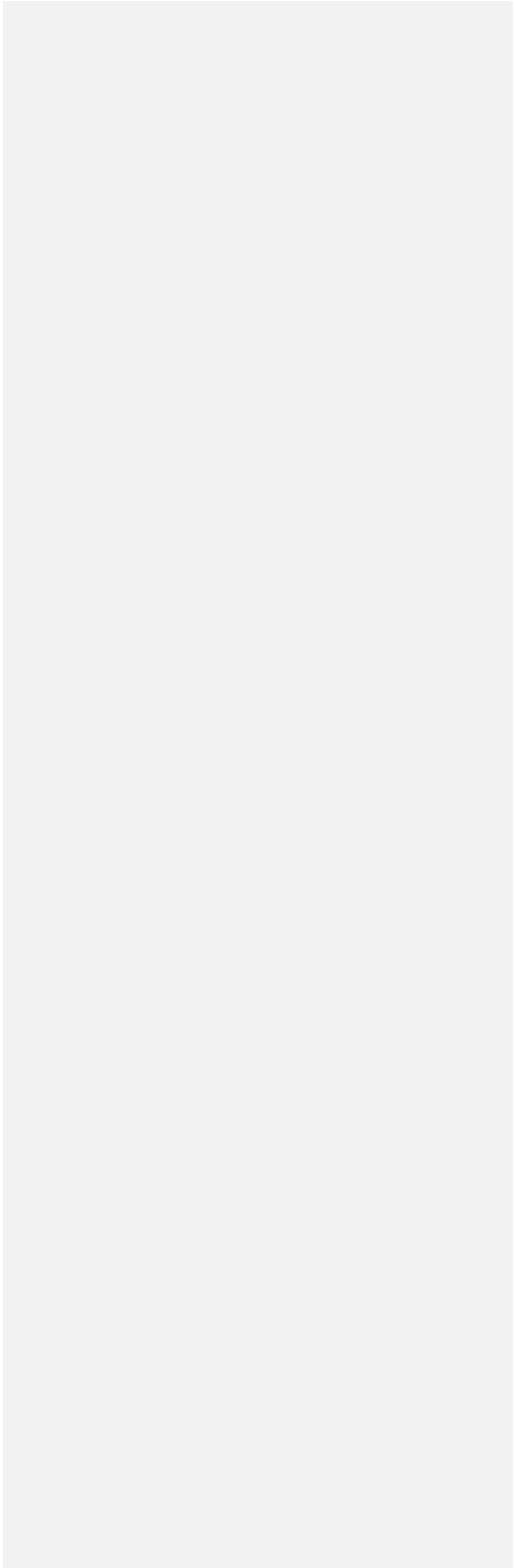
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Abstract

The use of swine has evolved far beyond their foundational agricultural purposes. Recent advancements in utilizing pigs as translational models for human medicine have significantly expanded our understanding of health and disease, not only in human medicine but also through an agrimedical lens. This thesis introduces a novel design of the 8-Arm Radial Maze to identify cognitive deficits and differences in blood biomarkers in Ossabaw pigs (CorVus Biomedical, Crawfordsville, IN; $n = 32$; boars and gilts), a well-established model of obesity, on either a healthy diet or a Western Diet. Additionally, this work advances previous research on physiological biomarkers by evaluating complete blood count and serum chemistry panels after 16 weeks.

Study 1 aimed to assess cognitive impairment in Ossabaw pigs fed a normal diet or a Western Diet, which is a hypercaloric, high-fat, high fructose corn syrup formulated diet. A newly designed 8-Arm Radial Maze, capable of accommodating swine up to 200 kg, was used to assess key domains of cognition: working memory, reference memory, operant conditioning, and spatial discrimination learning. After 9 weeks of starting their respective diets, pigs began the first phase, conditioning, which included six sessions for acclimation and training. For conditioning, pigs were required to locate eight rewards, one in a bowl within each arm. In the second phase, acquisition, cognitive tasks required pigs to identify a unique configuration of only two correct bowls out of 8 across 15 sessions. Obese pigs exhibited early signs of cognitive impairment during the conditioning phase, such as reduced maze completion. These deficits were more distinct in the acquisition phase, with obese pigs entering fewer correct pods, taking longer to locate rewards, and spending more time in areas such as the arms, bowls, and center, which are behaviors indicative of distraction and reduced task efficiency. This study provides evidence

that a pig model on the Western Diet can exhibit cognitive deficits as early as 9 weeks, potentially reflecting a potential nutrient and chronic inflammation effect on the brain.

Study 2 aimed to improve understanding of peripheral biomarkers in the same pigs as Study 1 for 6 and 16 weeks on a Western Diet. Few studies included hematological findings in obese Ossabaw pigs, and experimental design and pig type varied. Blood samples were collected before starting the diet (baseline, covariate) and again at weeks 6 and 16 in relation to starting their respective diets. Sampling was performed using a Panepinto sling, a low-stress method that minimizes the stressor of handling and restraint, and isoflurane application. Notable hematological and physiological changes emerged at 6 and 16 weeks after starting their diets. Significant differences were observed in lipid metabolism, inflammatory markers, and blood cell profiles, suggesting early signs of immune dysregulation and metabolic dysfunction.

Together, these studies conclude that the early stages of obesity are associated with both cognitive and hematological changes, highlighting a wide spectrum of physiological concerns linked to the Western Diet. In the face of a growing global obesity crisis and rising food insecurity, it is crucial to advance our understanding of obesity-related outcomes and emphasize the urgent need for early dietary intervention. Beyond biomedical relevance, these findings also carry important implications for the swine industry, where over-conditioning and metabolic disorders in breeding stock can impact productivity, longevity, and overall animal welfare. By bridging agrimedecine with applied animal science, this research underscores the value of using swine as a translational model that benefits both human health and livestock management practice.

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Chapter 1 - Review of Literature

Introduction

The growing prevalence of obesity poses significant challenges to both human and animal health, extending beyond metabolic dysfunction to a chronic, low-grade inflammatory state that affects multiple organ systems, including the immune system and brain. Odle et al. (2014) define agrimedical research as basic or applied investigation that simultaneously advances both agricultural and medical sciences by using animal models to explore conditions relevant to both humans and livestock. Agrimedical experiments can be used to 1) understand how obesity influences physiological and cognitive health before the pathological state in a relevant animal model for human health, and 2) may provide impactful information to swine producers about the effects of overfishing breeding stock, where physiological and anatomical similarities to humans allow for meaningful insights across both human medicine and animal agriculture.

Swine have emerged as exceptional translational models due to their similarities to humans in metabolic, cardiovascular, and immune systems, as well as brain structure and function (Gieling et al., 2010; Kornum and Knudsen, 2010; Lunney et al., 2021; Netzley and Pelled, 2023). Compared to non-human primates, pigs offer logistical advantages, including affordability, housing feasibility, and fewer ethical constraints, while still providing relevant data (Kornum and Knudsen, 2010; Cluzel et al., 2022).

By evaluating obesity-related inflammation and cognition in pigs, the work within this thesis seeks to contribute to both medical and agricultural knowledge, reinforcing the role of pigs in advancing agrimedical research. This literature review explores current findings on the

obesity-inflammation-cognition axis, neuroinflammatory mechanisms, and the growing value of pigs in bridging the gap between human and animal health.

Food Insecurity-Obesity Paradox

Food is fundamental to human wellbeing, and human development is not possible without food security. Despite global food production keeping ahead of global demand, still, one billion people are left without enough food to eat, and even more go without adequate nutrition (Pinstrup-Andersen, 2009; Godfray and Garnett, 2014). Food security refers to the availability of enough food at a variety of levels – global, national, community, or household (Pinstrup-Andersen, 2009). The main driver for food insecurity is the rapidly increasing human population. Since the 1950s, there has been a logarithmic increase in the world's population (United Nations, N.D). Despite food production currently keeping ahead of global demand, patterns in data suggest that this will become a major issue in the future (United Nations, N.D). The United Nations projects that the world's population will grow from 8 billion in 2023 to 9.7 billion in 2050 and could peak at nearly 10.4 billion in the mid-2080s before a predicted decline of -0.1% per year (United Nations, N.D).

Originally, food security was defined as a country's ability to meet dietary energy needs (Pinstrup-Andersen, 2009). In 1996, the World Food Summit defined food security as existing when all people always have physical and economic access to sufficient safe and nutritious food to meet their dietary needs and food preferences for a healthy and active lifestyle (FAO, 1996). Keywords within the statement stand for specific meaning – “safe and nutritious” refers to food safety and complete nutrition, while “food preferences” expands food security from having enough food to eat to having enough food to be preferred. When discussing food security at the national level, there is a distinct difference between availability and access. One country's

government may offer enough food, but access to food may not be guaranteed for all individuals (Pinstrup-Andersen, 2009). Additionally, enough calories do not ensure a healthy and nutritional diet. A household may be food secure if enough food is provided for all members of the household, but they undergo periods of insecurity, depending on seasonal shortages. In contrast, in a household with static food insecurity, the entire family experiences a long-term lack of access to adequate food (Pinstrup-Andersen, 2009). According to Misselhorn et al. (2012), pressures that can be applied to food insecurity on the supply side include climate change, food safety and nutritional value, globalization, and food prices. On the demand side, pressures include population increases, urbanization, changes in food preferences, and poverty (Pinstrup-Anderson, 2009; Misselhorn et al., 2012). These growing demands place immense pressure on a limited number of livestock species that currently supply the bulk of the world's animal protein (Roth and Gaylon, 2024).

Most of the world depends on three species for the majority of animal protein production: chickens for meat and eggs, cattle for milk and meat, and swine for meat (Roth and Gaylon, 2024). The dependence on these three species leaves animal protein production vulnerable to the emergence of diseases that can limit production (Roth and Gaylon, 2024). In addition, in many regions of the world, animal production within those species lacks genetic diversity, leading to a decline in quality and productivity (Neumann, 2002; Sufiyan, 2022; Roth and Galyon, 2024).

Although food insecurity is often associated with malnutrition and a lack of calories, food insecurity can also be seen in overweight people and households – a phenomenon known as the food insecurity–obesity paradox (Godfray and Garnett, 2014; Dhurandhar, 2016). The paradox may be driven by a lack of financial access to affordable, complete nutrition (Pinstrup-Andersen, 2009; Godfray and Garnett, 2014). This paradox may also be driven by the neurobiological

responses to perceived stress, where stress-related hormonal changes, such as chronically elevated cortisol, promote increased appetite, visceral fat deposition, leptin resistance, and impaired fat metabolism, ultimately leading to chronic positive energy balance and weight gain (Dhurandhar, 2016).

Obesity, once predominantly associated with upper-class households, is now increasingly prevalent in low- and middle-income populations (Godfray and Garnett, 2014; Roth and Galyon, 2024), contributing to a range of serious health outcomes including cardiovascular disease, strokes, diabetes, musculoskeletal disorders, gastrointestinal and respiratory issues, and even certain cancers (Haslam and James, 2005; Godfray and Garnett, 2014). The World Health Organization (WHO) reported in 2008 that 1.4 billion adults were overweight, and over 200 million men and nearly 300 million women were obese. During 2009–2010, the prevalence of childhood obesity was 16.9% in the United States (WHO, 2013).

According to the WHO, people with a body mass index (BMI) above 30 are considered obese, and people with BMIs between 25-29 are considered overweight. Although BMI is widely used to define overweight and obesity (Sweatt et al., 2024), this one-dimensional diagnostic tool is limited because it does not differentiate between fat mass and lean mass, nor does it reflect regional fat distribution such as visceral adiposity, which is more closely linked to metabolic and cardiovascular risk (Kolk et al., 2004). Body Mass Index alone may underestimate or misclassify individual health risk, particularly across age, sex, and ethnic groups, and it cannot fully explain phenomena like the obesity-food insecurity paradox (Gurunathan and Myles, 2016). Therefore, measures like waist circumference or waist-to-hip ratio, which better capture visceral fat, offer more clinically relevant assessments of obesity-related health risks (Kok et al., 2004).

Global food insecurity has worsened in recent years, particularly in low- and lower-middle-income countries, where over 25% and 17% of the population, respectively, now face severe food insecurity (FAO, 2024; Table 1.1). Simultaneously, these regions are experiencing rising rates of adult obesity and children who are overweight, reinforcing the complexity of the obesity paradox in the context of global food inequities (Neumann et al., 2022; FAO et al., 2024).

Despite advances in food production and global availability, there remains a critical gap in research into, and implementation of, procedures to ensure equitable access to nutritionally adequate food, especially for low- and middle-income populations. The paradox of obesity coexisting with food insecurity highlights a growing public health crisis, where energy-dense, nutrient-poor foods are often the most accessible and affordable options. Current strategies fail to fully address the intersection of economic hardship, dietary quality, and health outcomes. Thus, there is a pressing need for interdisciplinary research aimed at developing sustainable solutions, such as affordable, nutrient-dense foods or dietary supplements, which can bridge this nutritional divide and support both public health and food equity.

Obesity Related Behaviors

Stress is a significant driver of human eating behaviors, influencing food intake in both directions, undereating or overeating, depending on the severity and duration of the stressor (Torres and Nowson, 2007; Razzoli et al., 2017). Chronic life stress is associated with a greater preference for energy-dense, high-fat, and high-sugar foods (Torres and Nowson, 2007). Longitudinal studies suggest this “Western Diet” may causally contribute to weight gain. In addition to stress, environmental cues strongly influence human feeding behavior; studies show that humans are more responsive to changes in portion size, food variety, and social eating environments than to internal biological signals like energy depletion (Spitzer and Rodin, 1981).

Body weight fluctuations following major life changes, such as moving to a new environment, entering college, or changes in marital status, further highlight the dominant role of external factors in shaping eating behaviors (Spitzer and Rodin, 1981). Stress is also closely linked to disordered eating behaviors such as binge eating, with both laboratory and real-world studies demonstrating that individuals with binge eating disorders are more likely to binge during periods of acute stress (Razzoli et al., 2017). These findings suggest that stress and environmental factors, not just biological hunger cues, play a significant role in shaping human eating behaviors, which helps explain how individuals experiencing food insecurity, despite limited access to food, may still develop patterns of overeating, poor dietary quality, and ultimately obesity. Beyond effects on eating patterns, accumulating evidence indicates that obesity itself may alter cognitive function. Clinical and experimental studies have shown that obesity and high-fat feeding are associated with impairments in learning, memory, and executive function (Elias et al., 2003; Elias et al., 2005; Cournot et al., 2006; Sabia et al., 2009).

Rodent models have been instrumental in advancing our understanding of obesity's effects on behavior and cognition. In both rats and mice, stress-induced eating behaviors are well-documented, where consuming palatable foods helps alleviate negative emotional states and contributes to the development of obesity (McMillan, 2013). Analyses of feeding patterns, such as licking microstructure in rats, reveal that obesity alters both the motivation to eat and the evaluation of palatable foods (Johnson, 2018).

Beyond changes in ingestive behavior, cognitive impairments have been consistently observed in rodent models of obesity. Genetically obese Zucker rats exhibit deficits in memory performance compared to lean controls, suggesting that obesity may impair brain regions involved in learning and memory (Winocur et al., 2005). Similarly, mice exposed to high-fat

diets early in life demonstrate broad cognitive and emotional alterations, including increased anxiety-like behavior and vulnerability to depressive-like responses, particularly under stressful conditions (André et al., 2014; Yamada et al., 2011; Dinel et al., 2011).

Emerging evidence points to chronic low-grade inflammation as a key contributor to these behavioral and cognitive changes. In both diet-induced and genetic models of obesity, rodents show elevated expression of inflammatory markers in the brain, particularly in the hippocampus and hypothalamus, which are critical for cognitive and emotional regulation (Cani et al., 2009; Dinel et al., 2014; Erion et al., 2014).

Although rodent models have provided valuable insights into the behavioral and cognitive consequences of obesity, they have a greater phylogenetic distance to humans than large animal models, such as pigs. Nonetheless, pigs remain significantly underutilized in this area of research (van der Staay, 2006; van der Staay et al., 2009). The use of pigs as biomedical models is well established for cardiovascular, metabolic, and dermatological studies; however, their application in neurobehavioral and cognitive research is comparatively rare. A few studies in pigs have provided some insights but more research in mature animals with repeatable and reliable behavioral tests are needed. When pigs are used, studies have largely focused on Yucatan and Göttingen minipigs, primarily investigating early-life nutritional effects or diet composition rather than the direct cognitive consequences of obesity in adulthood (Val-Laillet et al., 2017; Gautier et al., 2018; Haagenen et al., 2013). While these studies suggest that dietary factors can influence cognitive development and behavior, they often do not fully model the chronic metabolic dysregulation seen in adult obesity. Thus, despite the translational potential of pigs, particularly given their similarities to human brain structure and metabolism, substantial gaps remain in understanding how obesity impacts cognition in this species. Collectively,

findings from human studies, rodent models, and limited pig research reveal that obesity consistently impairs cognition and behavior, suggesting that systemic inflammation may play a vital role.

Impact of Obesity on Peripheral Inflammation

While behavioral changes are an important consequence of obesity in both humans and animals, they often coincide with, and may be driven by, underlying physiological changes, including low grade, chronic peripheral inflammation. Obesity is defined as the excessive accumulation of fat within adipocytes, which ultimately contributes to chronic peripheral inflammation through the secretion of pro-inflammatory cytokines (Kopelman, 2000; Canello and Clement, 2006; Gregor and Hotamisligil, 2011). The inflammatory response associated with obesity is metabolically triggered, resulting from prolonged overconsumption of nutrients. Adipocytes, often referred to as metabolic cells, play a vital role in this process (Weisberg et al., 2003; Gregor and Hotamisligil, 2011). These cells sense nutrient excess and respond by initiating inflammatory signaling pathways. In other words, metabolic signals originating from adipocytes disrupt homeostasis and contribute to the onset of inflammation (Gregor and Hotamisligil, 2011). An additional feature of the inflammatory state caused by obesity is increased infiltration of macrophages (immune cells) into the metabolic tissues (Weisberg et al., 2003). In obesity, hypertrophic adipocytes often become surrounded by macrophages, forming crown-like structures that are a hallmark of inflamed adipose tissue (Altintas et al., 2011). Adipose tissue macrophages consist of at least two different phenotypes - classically activated M2 (i.e., resident) macrophages and alternatively activated M1 (i.e., recruited) macrophages (Fujisaka et al., 2009; Gregor and Hotamisligil, 2011). Recruited macrophages produce proinflammatory cytokines, such as TNF- α and interleukin-6 (IL-6), which contribute to the development of insulin resistance (Gordon, 2003).

In contrast, resident macrophages, which are the predominant macrophage population in lean adipose tissue, exhibit a distinct gene expression profile that is associated with tissue repair and the remodeling processes (Gordan, 2003; Lumeng et al., 2008). A study by Weisberg et al. (2003) showed that macrophages make up to 40% of all adipose tissue in obese mice, compared to 10% in lean mice. In addition to changes in their inflammatory profile, macrophages in obesity are highly adaptable to the surrounding lipid-rich environment (Lumeng et al., 2007; Castoldi et al., 2016; Matz et al., 2022). Through homeostatic mechanisms, macrophages activate lysosomal lipid metabolism, which allows them to process and store excess fats (Xu et al., 2013). This may be a natural way for the body to handle the elevated levels of lipids released by enlarged adipocytes during obesity (Castoldi et al., 2016).

Obesity is associated with detectable alterations in standard clinical blood panels, such as complete blood counts (CBC) and serum chemistry profiles, through physiological changes in circulation (Nimptsch et al., 2019; Jung et al., 2016). These panels offer clinically accessible indicators of systemic health, providing insights into metabolic status, organ function, and the presence of chronic low-grade inflammation (Nimptsch et al., 2019). Obesity-related disruptions can be reflected in blood markers associated with liver function, kidney function, cardiovascular health, and immune activity, offering an integrated view of the body's physiological response to chronic nutrient excess and adiposity (Jung et al., 2016). Monitoring these blood-based biomarkers provides valuable information about the systemic burden of obesity and can help identify individuals at risk for secondary complications. Thus, bloodwork panels serve as an important bridge between peripheral metabolic dysfunction and the potential development of central nervous system consequences, including neuroinflammation.

Impact of Obesity on Cognition and Neuroinflammation

Researchers widely acknowledge that obesity is associated with low-grade inflammation in peripheral tissues and the circulation (Gregor and Hotamisligil, 2011; Spencer, 2013). However, accumulating evidence suggests that obesity also results in inflammation in the brain. Peripheral inflammation can cause activation of microglia, which are macrophages of the brain. Clinical and experimental evidence indicated that obesity and high-fat diets are associated with deficits in learning, memory, and executive functioning (Elias et al., 2003; 2005; Cournot et al., 2006; Sabia et al., 2009). Inflammation, the body's defense response to harmful stimuli, is proposed to be an important mechanism underlying cognitive impairment and dementia (Gorelick, 2010). As a result, microglia are influenced by the peripheral pro-inflammatory cytokines that can infiltrate the blood-brain barrier due to increased permeability, ultimately leading to neuroinflammation, especially within the hippocampus (Posey et al., 2009; Miller and Spencer, 2014; O'Brien et al., 2017; Val-Laillet, 2019) which is a key region for learning and memory (Miller and Spencer, 2014; Val-Laillet, 2019). As a result, there is reason to believe that both systemic and central inflammation may converge to a final common pathway leading to not only impairment of hypothalamic regulatory pathways of feeding but also cognitive dysfunction (Miller and Spencer, 2014).

In addition to cytokine-induced neuroinflammation, other key aspects of the hypothalamus, the brain's neuroendocrine center, are negatively affected by obesity. The hypothalamus regulates food intake and energy balance by sensing insulin, leptin, and ghrelin, hormones involved in metabolic signaling. Obesity has a direct link to hypothalamic leptin resistance and, therefore, leptin is no longer able to properly suppress the appetite when appropriate (Posey et al., 2009). This leads to improper appetite regulation and, oftentimes, increased food consumption. Leptin resistance further contributes to hypothalamic neuroinflammation by promoting chronic activation

of microglia and astrocytes, leading to increased expression of proinflammatory cytokines. This creates a positive feedback cycle, where inflammation worsens leptin resistance, further impairing appetite control and promoting excessive food intake. Over time, persistent neuroinflammation contributes to neuronal damage, metabolic dysregulation, and cognitive impairments, highlighting the significant impact of obesity on hypothalamic function (Posey et al., 2009).

The Ossabaw Pig in Obesity Research

To better understand the link between obesity and physiological changes within the body, researchers have turned to the Ossabaw pig, a naturally selected pig that closely mirrors human metabolic and inflammatory pathways. This breed of pig gained its name from Ossabaw Island, located off the coast of Georgia (U.S.A). Ossabaw pigs exhibit unique metabolic characteristics that make it an ideal model for studying obesity and metabolic syndrome. They possess a thrifty genotype, believed to have conferred a selective advantage in environments with unpredictable and fluctuating food supplies for over 400 years (Hausman and Martin, 1981; Spurlock and Gabler, 2008; Sturek et al., 2015; Cluzel et al., 2022). This genotype enables efficient energy storage during times of abundance and improved survival during periods of scarcity (Sturek et al., 2015; Cluzel et al., 2022).

The Ossabaw pig is particularly well-suited for biomedical research on obesity due to its natural propensity for fat accumulation and its ability to develop hallmark features of metabolic syndrome, including insulin resistance and fatty liver disease, when exposed to a high-fat diet. In contrast, other commonly used breeds such as Yucatan and Göttingen minipigs offer advantages for growth and pharmacology studies but lack the same translational relevance for chronic, obesity-related diseases (Table 1.2). The Göttingen minipig, for example, is primarily used for

cardiovascular research; although it can be used in obesity studies, it requires a prolonged induction period and rarely develops fatty liver disease (Cluzel et al., 2022). The Yucatan minipig serves as an excellent surgical model but tends to develop only moderate obesity and insulin resistance, with the full metabolic syndrome phenotype emerging only with added hypertension (Cluzel et al., 2022). Commercial pigs are another option, as they develop metabolic syndrome rapidly and are widely available and cost-effective; however, the rapid onset of disease can limit study duration, and their large size requires substantial housing resources, making them more commonly used in neonatal models (Cluzel et al., 2022). Additionally, the Ossabaw pig surpasses other breeds in biomedical relevance due to its natural variation in body type, a result of natural selection, whereas breeds like the Göttingen and Yucatan minipigs are more homogeneous and were developed through artificial selection (Cluzel et al., 2022).

Ossabaw Pig Obesity and Biomarkers

Given the Ossabaw pig's genetic predisposition to fat accumulation and metabolic dysfunction, they serve as a highly relevant model for studying how dietary factors, such as a Western-style, Metabolic Syndrome (MetS) diet, drive the development of obesity and related physiological changes. The Western diet is intentionally formulated to be high in calories, fructose, and fat. This diet reliably induces features of metabolic syndrome, including abdominal obesity, insulin resistance, glucose intolerance, hypertension, and dyslipidemia, in genetically predisposed individuals (Kreutz et al., 2011; O'Neill and O'Driscoll, 2015). While environmental and genetic factors both contribute to the onset of metabolic syndrome, a calorie-dense diet and sedentary lifestyle remain central triggers in susceptible populations (Kreutz et al., 2011).

Numerous studies have utilized the Ossabaw pig as a translational model for Metabolic Syndrome, using a high-fat, high-fructose, high-cholesterol diet to induce the key symptoms of the condition. As summarized in Table 1.4, despite the considerable variation in age at diet initiation, sex, and duration of exposure, most studies report consistent elevation in blood glucose, cholesterol, and triglycerides. Ages ranged from juvenile pigs at 5 weeks old (Panasevich et al., 2018), to mature adults aged 6-8 years old (Newell-Fugate et al., 2014), with diet durations from as short as 9 weeks (Dyson et al., 2006), to as long as 64 weeks (Westover et al., 2016). While many studies did not specify sex, several studies focused exclusively on females (Dyson et al., 2006; Panasevich et al., 2018) and one on males (Alloosh et al., 2008). Although chemistry panels were commonly reported (including markers like glucose, triglycerides, and liver enzymes), complete blood count parameters were rarely evaluated. The exceptions were Kreutz et al. (2011), who reported increased platelet reactivity, and Matthan et al. (2018), who reported increased alkaline phosphatase and alanine transaminase in obese pigs.

Importantly, there is a lack of data on healthy reference ranges for Ossabaw pigs. Blood samples in Ossabaw pig studies are typically collected in the context of specific experimental objectives, often after animals have undergone dietary or surgical interventions. As a result, reference values for healthy, untreated Ossabaw pigs, especially differentiated by sex and age, are largely unavailable. In the absence of Ossabaw-specific baseline data, researchers often rely on values from other miniature pig breeds, such as the Yucatan pig, due to perceived physiological similarities. Existing bloodwork data from wild-caught or domestically raised adult male Ossabaw pigs (Sturek, 2016) provide limited insight due to the absence of gilts and lack of control over environmental variables. While some studies have included both sexes, such as Kleinbongard et al. (2022), all animals were subjected to experimental manipulation (e.g., induced ischemia),

making it difficult to isolate true baseline values. Even studies that report blood data from pigs on lean control diets, like Kreutz et al. (2011), often exclude sex-specific reporting, which introduces further uncertainty. The cumulative lack of comprehensive baseline values, including age- and sex-specific ranges, limits the interpretability of findings across the field and underscores the need for establishing reference standards that reflect the unique physiology of Ossabaw pigs.

Assessing Cognition using the 8-Arm Radial Maze

While blood biomarkers provide valuable insight into the physiological effects of obesity, there remains a critical gap in understanding how obesity impacts cognitive function in Ossabaw pigs, given the potential the breed has in translational metabolic research. To address this, cognitive assessment tools such as the 8-arm radial (8-ARM) maze offer a structured and repeatable approach to evaluating learning, memory, and decision-making in this model. This shift in focus brings us to the study of cognition, a key component in understanding how obesity may influence decision-making, memory, and overall brain function. Cognition can be broadly defined as the range of mental processes involved in acquiring, processing, and utilizing information from experiences, thoughts, and sensory input (Gielsing et al., 2010). This includes functions such as attention, memory, social learning, judgment, reasoning, and decision-making (Held et al., 2001; Bushby et al., 2018; Gielsing et al., 2010). To assess cognition, measures of learning and memory are quantified. Learning can be defined as the acquisition of knowledge and formation of memory, while memory can be defined as a recollection and the permanence of learning (Bimonte-Nelson, 2015). Working memory, a form of short-term memory, involves the retention of information for a short period, typically trial-specific information. Reference memory, on the other hand, is a form of long-term memory and is needed to retain information

consistently over time, such as task-specific details (Olton and Samuelson, 1976; Gieling et al., 2010; Bimonte-Nelson, 2015; Roberts et al., 2016).

In recent years, there has been a surge of interest in using minipigs for cognitive research due to several key factors. These factors include the support of biomedical translational research by comparing the pig brain to the human brain, and a better understanding of the pig's cognitive functions and welfare needs in industry (Gieling et al., 2010; Kornum and Knudsen, 2010). The cognitive abilities of pigs can be tested using a variety of tasks, paradigms, and methods (Table 1.3).

The 8-ARM might offer a structured, repeatable, and reliable approach to assessing learning, memory, and cognitive flexibility in swine. The 8-ARM was first introduced as a spatial maze paradigm based on the principle that rodents preferentially use spatial cues to solve discrimination tasks when a specific location is consistently associated with a reward (Olton and Samuelson, 1976). The 8-ARM apparatus has equally spaced arms radiating from a central arena that takes advantage of the pig's natural novelty-seeking and spatial foraging strategies. The goal of the maze is for the subject to remember which arms have accessible rewards (correct answers) and which have inaccessible rewards (incorrect). Therefore, the 8-ARM requires both working memory and reference memory. Task-specific details include the exact locations in which the food reward can be found (Gieling et al., 2010). By placing rewards in only some of the arms and keeping these arms consistent throughout the task, the 8-ARM can be used to assess both types of memory simultaneously (Bimonte-Nelson, 2015). Working memory errors occur when the animal revisits previously explored baited arms, while reference memory errors happen when the animal visits arms that are never baited (Bimonte-Nelson, 2015). Despite the wide variety of cognitive assessments available, the 8-ARM remains one of the most promising tools due to its ability to

evaluate multiple memory types while also allowing pigs to engage in natural spatial behaviors, enhancing its ecological validity (Olton and Samuelson, 1976).

Quantifying Cognitive Behaviors

The 8-ARM offers a variety of objectives and methods. With small adjustments, researchers can design experiments to assess reference memory, working memory, or both. In addition, small adjustments can be made to assess spatial learning and non-spatial learning. There are a variety of ways that researchers can quantify behaviors, some including the number of incorrect arm entries, latency to correct arm(s), total distance moved (Dilger and Johnson, 2010a; Fleming and Dilger, 2017), number of trials needed to learn the visual cue (Wang et al., 2007), time spent in the center, and time spent in arms (Fleming and Dilger, 2017).

Over the years, the methodology for analyzing these behaviors has evolved. Traditionally, researchers would take live observation notes with pen and paper and then conduct statistical analyses using their preferred software. As applied ethology has progressed, researchers (Puppe et al., 2007; Dilger and Johnson, 2010a; Fleming and Dilger, 2017; Nelson et al., 2023) have increasingly turned to time-coding tools such as ANY-Maze (Stoelting Co., IL, USA), Noldus Observer (Noldus, Wageningen, The Netherlands), or EthoVision (Noldus, Wageningen, The Netherlands) for coding behaviors, analyzing sequences, and integrating various data types. For these methods, the use of cameras is required to record all trials of the study, after which the videos are uploaded into the coding software. More recently, researchers have begun using machine learning (ML) and artificial intelligence (AI) to quantify behaviors. Studies by Allen et al. (2023) and Wutke et al. (2021) highlight how AI technologies are becoming increasingly important in behavioral research by providing automated, objective, and high-throughput alternatives to manual coding. These systems enhance the precision,

consistency, and scalability of behavioral data collection, allowing researchers to detect subtle patterns and complex behaviors that may be missed or inconsistently captured through traditional observation methods.

Reliability of Behavioral Sciences

In the context of behavioral maze research, reliability can be broadly defined as measuring animal performance consistently across a given maze task. Consistency in researchers' understanding of data collection techniques is essential for ensuring reliability, and equally important is the reliability of the software and tools used for analysis and quantification. Both researchers and software must be reliable with each other, within studies, as well as across studies, to minimize error and reduce variability in the data. To design and complete a cognitive maze task using animals, there must be a degree of reliability among researchers and the software being used (Bimonte-Nelson, 2015).

When assessing how well behavior is measured, both reliability and validity must be considered (Kaufman and Rosenthal, 2009; Bimonte-Nelson, 2015). Reliability refers to the consistency and repeatability of a measurement, where a reliable measure produces consistent results over time with minimal errors (Kaufman and Rosenthal, 2009). Factors such as precision (repeated results under the same conditions), sensitivity (ability to detect minor differences), resolution (the smallest detectable difference), and consistency (reliable results over time or across observers) can influence reliability (Kaufman and Rosenthal, 2009; Bimonte-Nelson, 2015; Rudeck et al., 2020). Validity, on the other hand, is whether a measurement accurately reflects the behavior it is intended to measure and provides relevant information to the research questions (Bimonte-Nelson, 2015; Rudeck et al., 2020). Key aspects of validity include accuracy (the measurement's closeness to the true value), specificity (how well the measurement targets

the behavior of interest without interference), and scientific validity (encompassing factors like construct, content, and criterion validity) (Meagher, 2009; Bimonte-Nelson, 2015). Together, reliability ensures consistency, while validity ensures that the measurement is meaningful and truly reflects the behavior being studied (Martin and Bateson, 2007). To design and complete a cognitive maze task using animals, there must be a degree of reliability among researchers and the software being used (Bimonte-Nelson, 2015). When creating an AI-based tracking system, establishing a reliable reference dataset with high observer agreement is essential for validation. Without a strong reference standard, AI-based analysis cannot be properly trained or evaluated, potentially compromising the reliability and validity of the measurements produced (Mathis and Mathis, 2020).

Conclusion

Obesity presents a growing threat to global health, marked not only by excess fat accumulation but by chronic, low-grade inflammation and emerging cognitive dysfunction. Through an agrimedical lens, swine models, especially the Ossabaw pig, offer critical insights into these early-stage disruptions, well before the full onset of metabolic syndrome. This thesis focuses specifically on the pre-pathological state of obesity, a stage often overlooked in research yet essential for identifying early interventions that may prevent irreversible physiological damage. By assessing both behavioral changes and peripheral and blood biomarkers in this early window, we aim to capture the subtle but significant shifts that precede overt disease. Although tools like the 8-arm radial maze have proven effective for evaluating cognition in swine, their application in pre-metabolic syndrome research remains limited. Furthermore, the lack of age- and sex-specific baseline blood data in Ossabaw pigs continues to hinder comparative analysis

and translational potential. Filling these gaps is vital for developing early, targeted strategies to mitigate the long-term consequences of obesity in both human and animal populations.

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Tables

Table 1.1 Human population percentages experiencing severe food insecurity between 2014–2016 and 2021–2023, alongside obesity and prevalence of overweight children in 2022, stratified by global region and income classification. Notably, food insecurity increased across all groups, with the most severe rates observed in low-income and the least developed countries. In contrast, obesity and rates of overweight children were highest in high-income and upper-middle income countries, highlighting the global paradox of coexisting food insecurity and obesity.

Region	% of Population Severely Food Insecure 2014-2016	% of Population Severely Food Insecure 2021-2023	% of Obese Adults 2022	% of Overweight Children 2022
World	7.6	10.9	15.8	5.6
Least Developed Countries	19.2	22.1	8.1	3.2
Low-income Countries	21.3	25.7	9.6	3.3
Lower-Middle Income Countries	12.0	17.6	12.1	4.1
Upper-Middle Income Countries	2.6	3.3	15.8	9.0
High-Income Countries	1.5	1.8	25.9	7.6

Adapted from FAO et al., 2024.

Table 1.2 Review of literature on the comparison of Pig Breeds for Biomedical and Translational Research. This table summarizes key traits, obesity susceptibility, and primary research applications of three commonly used pig breeds.

Breed	Strengths	Weaknesses	References
Ossabaw	+ Most promising model for metabolic syndrome (MetS) + Develops obesity, insulin resistance. Fatty liver disease + Strong translational value for adults + Natural selection made them “thrifty”	– Requires a long-term diet for MetS – Inflammation varies across studies – Body and head variability – Lacking in published behavior	(Kreutz et al., 2011; Matthan et al., 2018; Walker et al., 2019; Zhang et al., 2021; Cluzel et al., 2022)
Gottingen Miniature Pig	+ Commonly used in cardiovascular and obesity research + Homogenous body shape; small for human-based imaging + Gilts develop MetS traits more rapidly, like human trends + Published behavior studies	– Long induction period for full phenotype – Fatty liver disease rarely develops – Artificially selected for models other than obesity and MetS	(Larsen et al., 2007; Aigner et al., 2010; Cluzel et al., 2022)
Yucatan	+ Commonly used as surgical models and for intervention studies + Moderate obesity and insulin resistance + Some published behavior studies	– Mild phenotype without added hypertension – Weak cardiac pathology with diet alone – Lean growth	(Phillips et al., 1982; Otis et al., 2003; Larsen et al., 2007; Cluzel et al., 2022)

Table 1.3 Mazes and behavioral tasks commonly used to assess cognitive abilities in swine. Each task is associated with specific cognitive domains, such as spatial learning, memory, discrimination learning, social cognition, or long-term memory.

Task	Tested Cognitive Abilities	Author
T-Maze	Discrimination/recognition learning, spatial learning, and memory	(Tanida et al., 1991; Mendl et al., 1997; Nielsen et al., 2009; Elmore et al., 2012; McCrea, 2018; Allen et al., 2023)
Y-Maze	Discrimination/recognition learning, social discrimination/recognition learning	(Tanida and Nagano, 1998; Koba and Tanida, 1999; Kristensen et al., 2001; McLeman et al., 2005; McLeman et al., 2008)
8-Arm Radial Maze	Discrimination learning and memory, spatial learning and memory	(Laughlin et al., 1999; Laughlin and Mendl, 2000; Laughlin and Mendl, 2004; Wang et al., 2007; Dilger and Johnson, 2010b)
Three-choice point water maze	Spatial learning and memory	(Hammell et al., 1975)
Cognitive Holeboard	Spatial learning and memory	(Arts et al., 2009; Bolhuis et al., 2013; Haagensen et al., 2013; Antonides et al., 2015; Grimberg-Henrici et al., 2016; Roelofs et al., 2017)
Adjusted Hebb-William's maze	Learning ability and long-term memory	(Dejong et al., 2000)
Modified Morris water maze	Spatial learning and memory	(Siegford et al., 2008)
Mirror Task	Social cognition	(Broom et al., 2009)
Multi-Room Maze	Cerebral Dysfunction	(Hagl et al., 2005)

Table 1.4 Literature Review of Complete Blood Count and Chemistry Findings of Ossabaw Pigs on a High-Fat, High-Fructose Diet.

Study	Age	Sex	Sample Size	Duration on MetS Diet	CBC Findings	Chemistry Findings
Alloosh et al., 2008	~20 weeks	Boars	Not stated	55 weeks	--	↓ Creatinine, ↓ BUN
Chu et al., 2011	--	--	Lean: 9, Obese: 9	≥7 weeks	--	↑ Total cholesterol, ↑ Glucose
Dyson et al., 2006	5-6 Months	Gilts	Lean: 9, Obese: 8	9 weeks	--	↑ Glucose, ↑ Cholesterol, ↑ Triglycerides
Kreutz et al., 2011	6-7 Months	--	Lean: 10, Obese: 10	44 weeks	↑Platelet reactivity	↑ Cholesterol, ↑ Triglycerides
Neeb et al., 2008	--	--	MetS: 6	28 weeks	--	↑ Glucose, ↑ Triglycerides, ↑ Cholesterol
Panasevich et al., 2018	5 weeks	Gilts	Lean: 6/4, Obese: 6/4	16 week and 36 week cohorts	--	↑ Glucose (esp. 36 wk), ↑ Triglycerides, ↑ Cholesterol, ↑ ALP & GGT
Westover et al., 2016	24 weeks	--	--	64 weeks	--	↑ Glucose, ↑ Triglycerides, ↑ Cholesterol
Newell-Fugate et al., 2014	6-8 years	Sows	Lean: 5, Obese: 4	52 weeks		↑ Glucose, ↑ Triglycerides, ↑ Cholesterol
Matthan et al., 2018	5-8 weeks	Boars and Gilts	Lean: 8, Obese: 8	24 weeks	--	↑ Cholesterol, ↑ ALP, ↑ ALT

Chapter 2 - A Novel Approach to Cognitive Testing in Ossabaw Pigs:

Training and Utilizing the 8-Arm Radial Maze

Abstract

The objective of this study was to assess cognitive function in Ossabaw pigs (CorVus, Crawfordsville, IN; $n = 32$; boars and gilts; diets applied at 3.3 ± 0.52 months of age) using an 8-Arm Radial Maze (8-ARM; Hulbert and ShapeMaster, Ogden, IL). Pigs were assigned to a lean diet (5L80, Purina, IN) or an obesogenic diet (KT324, CorVus, IN) for 16 weeks. Two phases of the 8-ARM were designed to assess cognition (conditioning; 2 weeks (6 sessions); and acquisition; 5 weeks (15 sessions). In the conditioning phase, designed for acclimation, all 8 bowls had both inaccessible and accessible rewards (i.e., mini marshmallows). In the acquisition phase, designed for assessing learning and memory, each session used all 8 arms with inaccessible rewards in all 8 bowls and a unique configuration of 2/8 accessible reward bowls. The 8-ARM assessed working and reference memory, latency, accuracy, and reward-driven behavior. Behavior was recorded through live observation, video data, manual timecoding of videos, and a machine learning (artificial intelligence, AI) tracking system. In the conditioning phase, obese pigs had a smaller percentage of pod completion ($TRT \times Week$, $P \leq 0.001$), bowl completion ($P = 0.006$), and total maze completion ($P = 0.025$). Obese pigs also spent more time in the pods ($P \leq 0.001$) and at bowls ($P \leq 0.001$), in comparison to lean pigs. In the acquisition phase, obese pigs initially reached the maze center faster, but by later weeks, lean pigs entered more quickly ($P = 0.003$). Obese pigs spent more time in the center ($P = 0.004$), particularly during weeks 2 and 5. They also had longer latencies to enter both the first ($P \leq 0.001$) and second correct pods ($P = 0.046$) and were slower to locate the first ($P = 0.076$) and second rewards ($P = 0.005$). Obese pigs showed a higher frequency of repeated correct bowls ($P =$

0.027) and pods ($P = 0.013$) and had a greater ratio of incorrect to total pod entries ($P = 0.013$). These results suggest that obese pigs are not physically impaired, as indicated by initial center latency. However, they were less trainable, as seen in the conditioning phase. Similarly, in the acquisition phase, obese pigs displayed more distracted behavior (i.e., increased center duration) and exhibited cognitive deficits, including delayed reward discovery and increased repeated correct entries, indicative of working-memory errors. These findings support the hypothesis that obesity may impair cognitive function, potentially via neuroinflammatory pathways.

Keywords: Maze; Cognition; Neuroinflammation; Porcine; Ossabaw; Swine; Pig; Obesity; Obese

Introduction

Recent research has highlighted the link between obesity and cognitive impairments, particularly in relation to learning, memory, and executive functions (Elias et al., 2003; Sabia et al., 2009). Obesity triggers chronic low-grade inflammation, not only in peripheral tissues but also within the brain, contributing to cognitive dysfunction. This neuroinflammation is primarily driven by the activation of macrophages, which release pro-inflammatory cytokines that disrupt the blood-brain barrier and alter microglia function. Microglia are responsible for protecting the brain from injuries and diseases (Russo et al., 2022). Increased proinflammatory microglia can impact regions such as the hippocampus a key area for learning and memory (Posey et al., 2009; Val-Laillet, 2019) and the pre-frontal cortex, a key area of executive functioning (Jelsing et al., 2006). These interconnected processes underscore the significant impact of obesity and neuroinflammation on brain function and cognition, especially in regions critical for behavior and decision-making.

Cognition encompasses a broad range of mental processes used to acquire, process, and utilize information gathered from experience, thoughts, and sensory input. These cognitive functions include attention, memory, reasoning, judgment, decision-making, and social learning (Held et al., 2001; Gieling et al., 2010; Bushby et al., 2018). Cognitive assessments often focus on advanced behaviors such as learning and memory, where learning is the process of acquiring knowledge, and memory refers to the retention and recollection of previously learned information (Gieling et al., 2010; Bimonte-Nelson, 2015; Ede and Parsons, 2023).

The interest in using pigs (*Sus scrofa*) for cognitive research has surged in recent years, driven by their relevance to both biomedical and agricultural research. Despite the growing use of pigs in cognitive research, no studies to date have evaluated cognitive performance specifically in Ossabaw pigs, even though they are one of the leading large-animal models for studying metabolic syndrome and obesity-related disease (Cluzel et al., 2022). The lack of current research done on the cognitive performance of obese Ossabaw pigs represents a critical gap, as the Ossabaw pig presents a unique predisposition to obesity, insulin resistance, and systemic inflammation that provides an important opportunity to understand how metabolic dysfunction influences cognition.

Cognitive studies in pigs serve a dual purpose: advancing biomedical translation research by drawing comparisons between the pig and human brain, and enhancing understanding of the cognitive functions and welfare needs of pigs in the swine industry (Gieling et al., 2010; Kornum and Knudsen, 2010). Agrimedical research, a term coined by Odle et al. (2014), refers to research that simultaneously advances both agriculture and medical sciences by utilizing animal models to investigate challenges faced by both animals and humans. This research framework has grown in popularity due to the physiological and anatomical similarities between pigs and

humans, making pigs valuable models for agrimedical research (Gieling et al., 2010; Kornum and Knudsen, 2010; Lunney et al., 2021; Netzley and Pelled, 2023). Furthermore, the cognitive abilities of pigs are vital to livestock production, where their memory, learning, and problem-solving skills directly impact productivity and welfare. For instance, pigs need cognitive skills to navigate unfamiliar equipment, find food, and manage social interaction within group housing (Held et al., 2001).

One of the many tools for studying cognition in animals is the 8-arm radial maze (8-ARM). This spatial maze was originally developed for rodents and was based on the principle that animals use spatial cues to solve tasks associated with rewards (Olton and Samuelson, 1976). In the 8-ARM, animals must remember which arms contain rewards and which do not, testing their working and reference memory. Working memory (short term) involves active assessment and problem solving, while reference memory (long term) involves the recollection of previously learned information (Olton and Samuelson, 1976; Gieling et al., 2010; Bimonte-Nelson, 2015). Over the years, little advancement has been made to the 8-ARM, making it one of the more labor-intensive cognitive tasks, especially for pigs. The physical scale of the maze must be adapted for larger animals like pigs, adding logistical challenges related to arena construction, trial consistency, and subject management. Together, these limitations have historically constrained the 8-ARM's scalability and precision in cognitive research beyond rodent studies.

Historically, researchers interested in cognition often analyzed behaviors such as the number of incorrect entries, latency to correct answers, and total distance moved (Dilger and Johnson, 2010a; Fleming and Dilger, 2017). To quantify and analyze these measures, a variety of methods have been used. The most traditional method is manual observation paired with non-artificial intelligence (AI) software, such as Noldus Observer (Noldus Observer Leesburg, VA,

USA). However, with advanced technology such as artificial intelligence (AI) tracking systems, the 8-ARM could become much more efficient and less demanding for researchers, as we have seen in other types of cognitive assessments (Wutke et al., 2021; Allen et al., 2023).

A newly designed 8-ARM used in this study was built with a small footprint and offers ease of reproducibility, making it a versatile tool for future research across species in both biomedical and agrimedical fields (8-Arm Radial Maze; Hulbert and ShapeMaster, Odgen, IL, USA). The historically termed "arms" are referred to here as "pods" due to a structural redesign: long, extending arm-like corridors were replaced with compact, polygonal-shaped pods to reduce the maze's physical footprint and accommodate larger subjects. The objective of this study is to evaluate the effects of a 16-week Western diet, compared to a control diet, on cognitive performance in Ossabaw pigs, as measured using the 8-arm radial maze and a machine learning-based behavior tracking system during a conditioning and an acquisition phase. We had hypothesized that obese pigs would have increased latency-to-find correct answers, increased error-frequency, and slower entrance velocity.

Materials and Methods

All experimental procedures adhered to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (IACUC #4831). All procedures were reviewed and approved by the Kansas State University's Ethics Committee for additional oversight.

Animals, housing, and husbandry

Ossabaw pigs (n = 32; 16 gilts and 16 boars; Corvus Biomedical, LLC, Crawfordsville, IN) were used in two blocks (16 pigs per block), with each block consisting of pairs matched by body weight and sex. Sample size calculation was completed similarly to Nelsen et al., (2023), where the calculation was completed using variables from veterinary epidemiology methods

previously described (Martin et al., 1987). The calculator was set to an alpha of 0.05 and the power (1-beta) to 95. Each measure's standard error was entered, and the desired magnitude of difference between sexes for each measure was entered.

Each pair was randomly assigned (3.3 ± 0.52 months of age) a diet formulated to maintain leanness (LabDiet Mini-Pig HF Grower 5L80; Purina Test Diet, Richmond, IN) or a western diet (KT324; Corvus Biomedical, Crawfordsville, IN). Prior to the Western Diet and control lean diet being applied, all pigs were fed a standard grower chow (DuMOR Hog Grower; Purina, Richmond, IN). The lean diet (up to 1,650 KCal per day; LabDiet 5L80, Purina, MO) supports healthy growth and leanness, consisting of 9.9% fat (measured as ether extract), 70.3% carbohydrates, and 19.7% protein. In contrast, the western diet (KT324, CorVus Biomedical, IN) is a hypercaloric (up to 3,400 KCal Per day), high-fat, and high-fructose diet designed to induce obesity, with 40.8% of its carbohydrate-derived calories coming from high-fructose corn syrup, 42.9% of its fat-derived calories from sources such as ether extract, and a protein content of 16.9%. To minimize digestive upset, pigs assigned to the Western diet underwent a 9-day step-up period in which increasing proportions of the Western diet were gradually integrated into their original lean diet.

In the first block, the western diet was given in a mash form, which caused complications with the automated feeding system, so in the second block, the western diet was given in pellet form. At age 6 ± 0.52 SD months, pigs were transported for 8 hours to Kansas State University's Large Animal Research Center (LARC). At LARC, pigs were housed in a climate-controlled facility. Pens were 3.7 x 3.7 m and were fitted with a feeding station that was 1.6 x 1.2 m. Pigs received water ad libitum, were fed twice daily, and given multiple types of environmental enrichment, cycled in and out of pens on a schedule, such as Astros (EasyFix; SD,

USA), Lunas (EasyFix; SD, USA), fixed bells, and milk jugs. After arriving at LARC, pigs were given one week to acclimate to their new surroundings and housing systems before testing began.

Apparatus

An 8-ARM (Hulbert and ShapeMaster, Odgen, IL, USA) constructed with heavy-duty plastic was used for the cognitive assessment. Each of the 8 arms, or rather pods, measured 174 cm in length and 112 cm in width, radiating from a central octagonal platform with a diameter of 151 cm. All dimensions were carefully designed to facilitate ease of movement for the pigs. The pods were constructed in a tapered, polygon shape (Figure 2.1), in contrast to the historically rectangular arms from which the original "radial arm" maze design derived its name. The maze was 122 cm in height (Figure 2.1). The flooring was made of fitted rubber stall mats, designed for easy cleaning and to provide a non-slip surface. Each pod featured a two-chambered bowl (Hulbert and ShapeMaster, Ogden, IL, USA) mounted on the furthest wall (Figure 2.2) of the pod. Each bowl contained both an accessible and an inaccessible chamber, with a mini-marshmallow (fruit-flavored) always placed in the inaccessible chamber to control for olfactory bias. In the correct pods, the accessible chamber contained a mini-marshmallow reward. Correct pods were illuminated with a green light, while incorrect pods were illuminated with a red light, which were also within the inaccessible chamber. The lights were used to provide a potential detection method for the machine learning algorithm to detect a correct vs. Incorrect answer. For the pigs, each pod featured a unique yellow sticker pattern to further aid the pig in associating visual cues with the reward locations.

Handling and Maze Application

The maze was located just through two sets of double doors from the pigs' housing area. Two trained students utilized walkie-talkies to communicate while handling and moving pigs to and from the maze. Handlers used hog boards to gently guide each pig from its home pen, out through double doors, and into the testing area. The holding area was a gated space just before the entrance to the maze. From here, the two trained technicians moving the pigs would then communicate via walkie-talkie with a third technician, the live observer, who was located within the testing area. One pig entered the maze at a time, and the order of pigs remained the same for the entirety of the study. All bowls were sanitized between pigs until they were clear of remaining saliva, in addition to the maze floor if soiled with excrement or urine.

Timeline and experimental paradigms

For this portion of the analysis, pigs were exposed to the 8-arm radial maze 21 times over 7 weeks. Maze trials were conducted on Mondays, Tuesdays, and Wednesdays, before morning feeding. The first 2 weeks (e.g., 6 sessions) were the conditioning phase, and the remaining 5 weeks (e.g., 15 sessions) were the acquisition phase (Figure 2.3). The conditioning phase allowed the pigs to habituate to the maze and to understand the goal of the task. For sessions 1-3, pigs were given up to 300 seconds to locate all 8 rewards. In addition, the lids of the bowls were open, exposing the reward and light without requiring the pigs to lift the lids. In sessions 4 and 5, pigs were given up to 180 seconds to locate all 8 rewards, and lids remained open. In the final conditioning sessions, pigs were given up to 180 seconds to locate all 8 rewards, but this time, lids were closed, requiring the pigs to lift the lids manually to locate and consume the rewards. Cut-off times were calculated based on a conditioning task previously studied (Nelson et al., 2023). After the time limit, pigs that refused to enter the pods were shaped using a stick baited

with one large marshmallow into the unentered pods to train them. The acquisition phase was designed to fully understand the cognitive capabilities of the pigs by randomly assigning them each a unique configuration of 2 dedicated correct pods, while the remaining 6 would remain incorrect (Figure 2.4). Pods 4 and 8 were never used in configurations, as pod 8 was designated for entry and exit of the maze, and pod 4 for symmetry purposes (Figure 2.4). For sessions 1-9, pigs were given up to 180 seconds to locate their 2 rewards, and this time was reduced to 60 seconds for sessions 10-15. Lids of the bowls remained closed during the entirety of the acquisition phase. The continuous reduction of time allowance allowed researchers to increase difficulty as pigs improved, enforcing an increase in cognitive demand. In the acquisition phase, shaping only occurred after 60 seconds if the pig failed to complete the task (e.g., locating both rewards). To account for time limit changes within statistical analysis, duration measures were calculated as a percentage. If both rewards were located before the allotted time expired, the pig was guided out of the maze, indicating session completion.

Live Observation and Video Recording

Both live observational data and video footage were taken from each session. A customized network video recorder (NVR) recorded each session continuously at 30 frames per second at a video resolution of 1080 pixels. A single bullet camera (GeoVision Model GV-EBL4702-2F; Geovision, Irvine, CA) was mounted at 90 degrees toward the maze to capture the entirety of the session. To keep note-taking strategies consistent across several technicians, it was deemed that when the pigs' ears crossed the threshold, or distinct floor lines separating the pods from the center, the student could then consider the pig had entered a different area of the maze (Figure 2.5). Other trained technicians who were handling and moving pigs to the maze

area would then communicate with the student, taking live observation notes to ensure the safety of the pigs and the effectiveness of the trials.

Manual Time Coding

Using live observation notes, six trained applied ethologists conducted behavioral coding in Observer 11.3 (Noldus Observer, Leesburg, VA) to establish a reference dataset without the use of AI. Observer reliability was assessed using Cronbach's alpha, with an average alpha of 0.9958 and an average confidence range between 0.993-0.9975. An ethogram was constructed within Noldus Observer, where entry into maze pods was classified as a state, and non-nutritive oral behavior (NNOB) directed toward the bowls was classified as an event (Table 2.1). Once inter-observer reliability was confirmed, students manually coded a total of 440 maze trial videos, again using Noldus Observer. This manually coded dataset served as the reference dataset for later comparison with machine learning (ML) output. Machine Learning Training and Data Extrapolation

Following the reference dataset creation, students annotated a total of 2,820 frames of recorded maze footage to train an AI detection and tracking system designed to analyze the pigs' spatial-temporal behavior (Naiel et al., 2025). Annotations focused on identifying the location of each pig's head, the floor of each pod, visual cues, and thresholds separating pods. The detection of the lights located within each bowl was not annotated because, as pigs became efficient in checking bowls, they would open the bowl just slightly, and the lid would stay closed. The system was developed using YOLOv8 for object detection and ByteTrack for multi-object tracking, providing robustness to missing and false detections. Once fully trained, the ML algorithm enabled automated assessment of learning and memory performance, which we would use for data analysis. In all methods of data collection and analysis, behaviors recorded included

duration, latency, and frequency. Duration refers to how much time, in seconds, was spent in each area of the maze, including pods, the center, and near bowls. Latency refers to how long it took the pig, in seconds, to get to each location within the maze, including pods, the center, and bowls. Frequency refers to how many times the pig entered or interacted with each part of the maze.

Statistical Analysis

Prior to data analysis, the data were organized into trials, sessions, and weeks for systematic quantification. Each week consisted of three sessions (Monday, Tuesday, and Wednesday), which together formed one trial week. Within each trial, each day was treated as a separate 'sub-session' for analysis purposes. All statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC). Data were first checked for normality using the Shapiro-Wilk test in the UNIVARIATE procedure. If normality assumptions were violated, data transformations including logarithmic, root, inverse, or arcsine transformations were applied. If assumptions remained unmet, non-parametric alternatives such as the Kruskal-Wallis test were used. For repeated measures analyses, the following covariance structures were considered: first-order autoregressive [AR(1)], heterogeneous autoregressive [ARH(1)], first-order ante-dependence [ANTE(1)], and compound symmetry (CS). The best-fitting covariance structure was selected using the corrected Akaike's information criterion (AICC). The subject effect was defined as Pen (pig ID), with two pigs housed per pen. To account for within-pen correlation and repeated measurements across time, residual maximum likelihood (REML) estimation was used. The Satterthwaite approximation was applied to calculate degrees of freedom, accounting for pen-to-pen variation. The model included treatment (TRT), block, week, and their interactions as fixed effects. Sex was included as a covariate, and pig ID nested within

pen was specified as a random effect. The session nested within the week was treated as a repeated measure. Least square means (LSMeans) with Tukey's adjustment were used for multiple comparisons. Non-significant individual fixed effects were removed from the model to improve model fit. A treatment difference of $P \leq 0.05$ was considered significant, and tendencies were considered if the P -value was between 0.05 and 0.10 after biological relevance was considered.

Results

Conditioning

Obese pigs spent more time (duration) in the entry zone (Pod 8) than lean pigs for weeks 1 and 2 (TRT*Week $P = 0.005$; Table 2.2; Figure 2.9a). Lean pigs had the least time spent in the center for week 1, but increased duration in the center for week 2 (TRT*Week $P = 0.007$; Table 2.2; Figure 2.9b). Obese pigs tended (TRT $P = 0.089$) to have less latency to reach the first pod than lean pigs (Table 2.2). Compared to their week 1 performance, lean pigs had increased latency to last bowl in week 2 (TRT*Week $P \leq 0.05$; Table 2.2; Figure 2.7), but obese pigs did not differ between weeks. Obese pigs had the least total maze, bowl, and pod completion in week 2 compared to lean pigs (TRT*Week $P \leq 0.05$; Table 2.2; Figure 2.6). Lean pigs had the least time spent in the maze (duration) on week 1 (TRT*Week $P = 0.005$; Table 2.2; Figure 2.8a). Obese pigs had spent more time (duration) at the bowls and in pods for both weeks compared to lean pigs (TRT*Week $P \leq 0.001$; Table 2.2; Figure 2.8). Obese pigs tended (TRT*Week $P = 0.067$) to have less of a maximum latency-to-leading than lean pigs for both weeks (Table 2.2).

Acquisition

Obese pigs spent more time in the maze than lean pigs (TRT, $P = 0.007$; Table 2.3). Obese pigs reached the center faster (latencies to center) than lean pigs in week 1 of acquisition (TRT*Week, $P = 0.003$; Table 2.3; Figure 2.10A). Obese pigs spent more time in the center than lean pigs in weeks 2 and 5 (TRT*Week, $P = 0.004$; Table 2.3; Figure 2.10B). Obese pigs had greater latencies to first and second correct pods than lean pigs in weeks 2, 4, and 5 (TRT*week, $P \leq 0.05$; Table 2.3; Figure 2.11A; 2.11B). Obese pigs had greater latencies to the first correct bowl in weeks 2, 4, and 5 (TRT*Week, $P \leq 0.05$; Table 2.3; Figure 2.11C; 2.11D). Overall, obese pigs had greater latencies to the second correct bowl than lean pigs (TRT $P = 0.001$; Table 2.3).

At week 5, obese pigs tended (TRT*Week, $P = 0.058$; Table 2.3) to spend less time (duration) at correct bowls and at week 4, more time at the incorrect bowls than lean pigs (TRT*Week, $P = 0.025$; Table 2.3; Figure 2.12B). Obese pigs had a greater frequency of re-entering the correct pods in weeks 1 and 3 than lean pigs (TRT*Week, $P = 0.005$; Table 2.3; Figure 2.12A). Overall, obese pigs revisited the correct bowl more frequently than lean pigs (TRT, $P = 0.026$; Table 2.3). Lean pigs had a greater total correct bowl frequency than obese pigs at week 5 (TRT*Week, $P = 0.043$; Table 2.3; Figure 2.12D), as well as a greater correct pod frequency at weeks 4 and 5 (TRT*Week, $P = 0.001$; Figure 2.12C). Subsequently, lean pigs had a greater ratio of correct: total pods at week 5 (TRT*Week, $P = 0.005$; Table 2.3).

Discussion

This study aimed to assess how 16 weeks on the Western Diet would affect the cognitive ability of Ossabaw pigs. Currently, there are no known studies done on the effects of a high-caloric, high-fructose corn syrup, high-fat diet on cognition using pigs as a model. Experimental

obesity has been shown to impair cognitive function in rodent models, with high-fat diets reducing memory, synaptic plasticity, and neurovascular function (Lynch et al., 2013). These effects are linked to hippocampal apoptosis and disrupted blood–brain barrier integrity (Li et al., 2013), which may contribute to cognitive decline and neurodegenerative risk (Gorelick et al., 2011). Therefore, this current work provides some foundation information on how the western diet influences measures of conditioning and learning in Ossabaw Pigs.

Conditioning

In both rodent and the limited pig 8-ARM tests, an evaluation of conditioning is not included. In the current review, the subjects were not conditioned prior to receiving dietary treatments, due to logistic restraints (i.e., the Source company does not have an 8-ARM or technicians to collect the data). Therefore, the conditioning stage in the present study is important to report. Laboratory animals are often subjected to environmental stressors, which can comprise welfare, physiology, psychology, and ultimately, the quality of data (Bailey, 2018; Jønholt et al., 2021). Training provides predictability and controllability, reducing stress, anxiety (Bassett and Buchanan-Smith, 2007; Christianson et al., 2008), and the use of positive reinforcement can enhance positive emotions and build trust between trainers and pigs (Poole, 1997; Lambeth et al., 2006). Therefore, it is important to provide a conditioning phase to allow for proper training and acclimation to the maze.

During the conditioning phase, differences in how lean and obese pigs adapted to the new maze environment and learned the rules of the task became evident. Obese pigs spent more time in the entry zone (pod 8) than lean pigs in both weeks one and two, but the time decreased for both treatments in the second week. This, consequently, increased obese pig latencies to locate the first correct pod. Spending more time in the entry zone before exploring the maze may reflect

obese pigs' lack of ability to acclimate and explore a novel area. This finding was seen in rats, where a high-fat diet reduced exploratory behaviors (i.e., decreased activity) and working memory in a Y-maze (Jurdak et al., 2008). For the current experiment, similar results were found for time spent in the center of the maze during the first week. By the second week, obese pigs reduced their time in the center, which was less than lean pigs. Boomhower et al. (2013) reported a similar finding in obese Zucker rats, where over time, they were more impulsive and spent less time discriminating between choices than their lean counterparts.

Obese pigs showed decreased latencies to their final correct pod and bowl; these shorter times likely reflect reduced overall maze completion, due to spending more time within pods or at bowls, running out of time before reaching additional rewards. Although they did not complete the maze at the same rate as lean pigs, they had greater total maze durations, supporting the interpretation that they required more time to locate all eight rewards compared to lean pigs.

Obese pigs had less maximum latency to human intervention using a baited stick (used for guidance when pigs refused to participate) than lean pigs. This finding is an indirect measure of the pig showing refusal behaviors to enter or leave an area in the maze during conditioning. Jønholt et al. in 2021 reported that it is imperative that subjects have minimal fear of a novel test arena, therefore, more research may be needed to determine if obese pigs have reduced novelty seeking. Healthy pigs tend to be novelty seeking and have more overall activity when presented with novel objects (Zeltser et al., 2020).

Acquisition

During the acquisition phase, clear differences between treatment groups emerged over time within the 8-ARM. In contrast to the conditioning phase, the acquisition phase provides

researchers with a longer-term perspective on cognitive changes occurring in Ossabaw pigs as obesity progresses.

Again, in the acquisition phase, the pig was guided out of the maze upon completion, when they either found their two assigned rewards or ran out of time. Obese pigs spent more time in the maze than the lean pigs, indicating that they were slower to complete the task or did not finish at all. Surprisingly, obese pigs reached the center of the phase faster than lean pigs in the first week of acquisition, which might suggest that although obese pigs were undergoing diet-induced obesity, their performance was not likely limited by physical mobility or motor deficits.

Obese pigs spent more time in the center of the maze in weeks 2 and 5, which may indicate a lack of exploratory behavior, or even signs of refusal, similar to what was found in the conditioning phase. Obese pigs consistently took longer to locate the correct pods and bowls, particularly in weeks 2, 4, and 5. Although their performance showed modest improvement over time, lean pigs demonstrated a more substantial and consistent reduction in latency, reinforcing the idea that obese pigs experienced greater difficulty with cognitive engagement throughout the acquisition phase. Latency measures are commonly used to evaluate spatial learning, cognitive engagement, and reference memory in maze-based tasks (Cordner & Tamashiro, 2015).

Obese pigs tended to spend less time at correct bowls, especially during week 4, which might indicate impaired satiety or reduced reward sensitivity. This behavior aligns with findings in rodent models, where obesity-prone mice demonstrated higher motivation for palatable food consumption compared to obesity-resistant mice, even before developing obesity (Inbar et al., 2020). Similarly, Berthoud (2012) reported that rats fed high-fat diets exhibited disruptions in normal gut-derived satiety signaling, which could explain why obese pigs did not feel as satisfied even after locating a reward, leading them to leave their correct bowls quickly and continue

searching. On the other hand, obese pigs spent more time at incorrect bowls during week 5, possibly reflecting a failure of inhibitory control, a concept described by Davidson et al. (2005) when rats were unable to suppress outdated reward memories and continued responding as if a reward might still be present. This could also explain why obese pigs re-entered correct pods and re-checked correct bowls more frequently than lean pigs. Re-entering already checked pods and bowls has also been defined as a direct error of working memory, as obese pigs were not as likely to recall which pods they had already entered during that session (Olton and Samuelson, 1976; Bimonte-Nelson, 2015). Lean pigs entered more total correct pods, checked more correct bowls, and exhibited a higher correct-to-total-pod ratio, which may indicate superior reference memory compared to obese pigs. These findings suggest that lean pigs demonstrate enhanced reference memory capacity, allowing them to recall previously learned reward locations across sessions more effectively than their obese counterparts, despite the potential to also exhibit working memory errors.

Conclusions

In summary, performance in the 8-ARM revealed clear cognitive and behavioral differences between lean and obese pigs, emerging as early as the conditioning phase and becoming more pronounced during acquisition, which may indicate neuroinflammation. These patterns were not due to physical limitations but rather reflect early cognitive impairments associated with diet-induced obesity as early as nine weeks on a Western Diet. Nonetheless, these findings highlight the utility of the Ossabaw pig as a translational model for obesity-related cognitive dysfunction and underscore the broader impact of obesity on learning, motivation, and problem-solving. These results not only inform biomedical and agricultural research but also

contribute to the advancement of machine learning–based tracking systems in both laboratory and commercial settings.

Implications

While the 8-arm radial maze is unlikely to be used directly in commercial swine settings, it serves as a valuable tool for advancing our understanding of pig cognition and behavior within the industry, where over-conditioned animals remain a concern. Improvements in dietary management, restriction protocols, and consistent body condition scoring practices are essential to support both animal health and welfare, as well as the use of effective dietary interventions to mitigate the side effects of obesity. Additionally, the integration of AI into the swine industry offers potential improvement in animal welfare and production efficiency, which is crucial as the global demand for animal-based products rises (Neethirajan, 2023; Sharifuzzaman et al., 2024). We have already seen some use of AI tracking systems in the swine industry for purposes of detecting stress (Neethirajan, 2023), estrus (Sharifuzzaman et al., 2024), social interaction (Wutke et al., 2021), and even disease (Ryu and Tai, 2022).

In terms of human medicine, results here imply that obesity might cause cognitive deficits, both short-term and long-term. In communities and households where access to nutritious food is limited, identifying sustainable and affordable dietary solutions is critical to potentially mitigate the negative health impacts of obesity prior to the pathogenic state.

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Tables

Table 2.1 Ethogram used on Noldus Observer XT (Observer 11.3; Noldus Observer Leesburg, VA) for manual, non-artificial intelligence behavioral coding in the 8-Arm Radial Maze.

Behavior	Definition	State or event	Code
Entrance (default)	Both ears in entrance	State	8
Center	Both ears in center	State	0
Pod1	Both ears in P1	State	1
Pod2	Both ears in P3	State	2
Pod3	Both ears in P3	State	3
Pod4	Both ears in P4	State	4
Pod5	Both ears in P5	State	5
Pod6	Both ears in P6	State	6
Pod7	Both ears in P7	State	7
Bowl1	Head over bowl1	Event	z
Bowl2	Head over bowl2	Event	x
Bowl3	Head over bowl3	Event	c
Bowl4	Head over bowl4	Event	v
Bowl5	Head over bowl5	Event	b
Bowl6	Head over bowl6	Event	n
Bowl7	Head over bowl7	Event	m
Bowl8	Head over bowl8	Event	l

Table 2.2 8-Arm Radial Maze Performance from Obese and Lean Ossabaw Pigs (n=32, gilts and boars, pair housed) in the Conditioning Phase. Obese pigs were fed a high-fat, high-fructose corn syrup diet, and lean pigs were on a standard chow for 9 weeks before cognitive testing. If refusal occurred, a penalty of 280s was added to the latency measures.

	Week 1						Week 2			P-Values	
	Lean	Obese	SEM	Lean	Obese	SEM	Lean	Obese	SEM	TRT	Week*TRT
Maze Completion, %	83.1	77.5	2.45	88.1 ^a	78.2b ^b	3.02	81.5 ^c	73.6 ^b	3.00	0.119	0.025
Reward Retrieval, %	82.8	76.7	2.53	87.7 ^a	77.9b ^b	3.12	80.4 ^b	73.1 ^c	3.10	0.099	0.006
Pod Completion, %	83.5	78.3	2.41	88.5 ^a	78.5b ^b	2.94	82.5 ^b	74.2 ^b	2.92	0.144	0.001
Latency to Center, s ¹	2.7	2.6	0.72	3.5	1.8	0.87	3.5	1.8	0.87	0.940	0.834
Latency to First Bowl, s ¹	8.1	10.7	0.96	8.8	7.4	1.42	12.5	9.0	1.21	0.210	0.824
Latency to Last Bowl, s	145.3	165.6	4.73	160.8 ^a	129.9 ^b	7.84	200.7 ^c	130.4 ^b	4.84	0.005	0.003
Latency to First Pod, s	6.8	9.0	0.91	7.6	6.0	1.37	10.7	7.4	1.11	0.089	0.517
Latency to last Pod, s	143.0	160.1	4.51	157.7 ^a	128.3 ^b	7.70	188.5 ^c	131.7 ^b	4.79	0.012	0.038
Total Maze Duration, % of session ²	90.9	95.3	1.22	87.6 ^a	94.1 ^b	1.79	94.0 ^b	96.6 ^b	1.34	0.005	0.005
Time Spent at Bowls, % of session	45.0	43.0	1.76	40.1 ^a	50.0 ^b	2.34	36.1 ^a	49.9 ^b	2.34	0.426	0.0001
Time Spent at Pods, % of session	66.0	63.8	1.76	60.0 ^a	72.1 ^b	2.29	56.3 ^a	71.4 ^b	2.17	0.383	0.0001
Time Spent in Entry Zone, s	8.6	7.3	0.79	7.0 ^a	10.3 ^b	1.36	5.2 ^a	9.5 ^{a,b}	1.34	0.252	0.005
Time Spent in Center Zone, s	19.5	27.1	1.27	18.6 ^a	20.4 ^a	1.73	30.2 ^b	24.0 ^{a,b}	1.71	0.0002	0.007
Minimum Latency to Leading ^{3,4} , s	42.4	46.6	4.13	24.9	60.0	7.59	27.2	66.0	7.25	0.475	0.840
Maximum Latency to Leading ⁴ , s	122.8	139.0	8.10	160.5 ^a	85.1 ^b	11.67	198.4 ^a	79.6 ^b	11.54	0.162	0.067

^{a,b,c,d} Using the Tukey-Kramer adjustment, LS Means differ ≤ 0.05 ; Some P-values were generated from ¹ Inverse transformed data, ² Arcsin transformed data, and ³ Log-transformed data. ^{1,2,3} Raw data are presented with transformed SEM. ⁴ When a pig displayed resistance to entering a new area, a technician with a baited stick would lead the pig into the center or adjacent pod.

Table 2.3 8-Arm Radial Maze Performance from Obese and Lean Ossabaw Pigs (n=32, gilts and boars, pair housed) in the Acquisition Phase. Obese pigs were fed a high-fat, high-fructose corn syrup diet, and lean pigs were on a standard chow for 11 weeks before cognitive testing.

	Lean	Obese	SEM	P-Values	
				TRT	Week*TRT
1st correct pod latency, s ¹	35.9	55.5	7.06	0.160	0.0001
2nd correct pod latency, s ¹	106.6	143.9	6.98	0.001	0.046
1st correct bowl latency, s ¹	37.5	58.7	7.23	0.047	0.008
2nd correct bowl latency, s ¹	111.0	146.9	7.35	0.001	0.191
Latency to center, x30 frames per s ²	0.9	0.8	0.08	0.171	0.003
Correct Bowl Frequency, no./session	1.9	1.7	0.10	0.293	0.043
Frequency of incorrect bowls, no./session	4.3	4.1	0.19	0.491	0.800
Frequency of all bowls, no./session	6.2	5.9	0.21	0.240	0.834
Correct: Total Bowl	0.3	0.3	0.02	0.293	0.248
Incorrect: Total Bowl	0.7	0.6	0.02	0.293	0.279
Repeated Correct Bowl Freq, no./session	0.2	0.4	0.05	0.026	0.272
Correct Pods Frequency, no./session	1.7	1.6	0.09	0.172	0.001
Frequency of Incorrect Pods, no./session	3.2	3.3	0.18	0.690	0.894
Correct: Total Pod	0.4	0.4	0.02	0.173	0.005
Incorrect: Total Pod	0.6	0.6	0.02	0.173	0.005
Repeated Correct Pod Freq, no./session	0.1	0.1	0.03	0.812	0.005
Duration at Correct Bowl, % of session ¹	11.5	9.9	0.63	0.133	0.058
Duration at Incorrect Bowl, % of session	17.6	19.5	1.31	0.295	0.025
Duration in Correct Pods, % of session	15.6	15.2	1.00	0.789	0.186
Duration in Incorrect Pods, % of session	27.9	31.1	1.89	0.249	0.501
Duration in Entrance, % of session	5.3	6.1	0.55	0.327	0.666
Duration in Center, % of session	24.3	31.9	2.36	0.032	0.004
Maze Completion, %	67.8	78.4	2.62	0.007	0.676

LS Means differ ≤ 0.05 ; Some P-values were generated from ¹Log transformed data; ²Inverse transformed data; ^{1,2} Raw data are presented with transformed SEM. ⁴When a pig displayed resistance to entering a new area, a technician with a baited stick would lead the pig into the center or adjacent pod.

Figures

Figure 2.1 An overhead schematic of the 8-arm radial maze (Hulbert and ShapeMaster, Ogden, IL, USA) used for cognitive testing in lean and obese Ossabaw pigs (n = 32; gilts and boars; Corvus Biomedical, LLC, Crawfordsville, IN). Each arm measures 173.9 cm in length and 111.7 cm in width, radiating from an octagonal center platform with a diameter of 151.1 cm. The design accommodates pigs up to 200 kg, allowing ease of movement and access during testing.

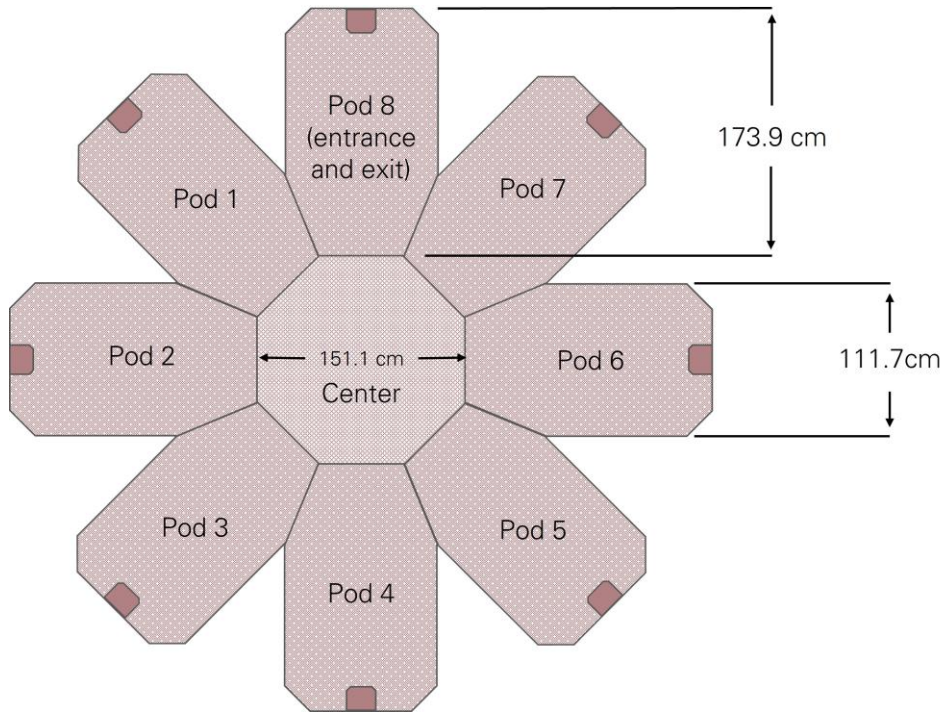


Figure 2.2 The MazeShape bowl (Hulbert and ShapeMaster, Ogden, IL, USA), used within the 8-arm radial maze for cognitive testing, was fastened to the inside of each pod on the furthest wall. The bowl consists of two compartments: one accessible and one inaccessible. To control for olfactory cues, an inaccessible reward was consistently placed in the lower compartment, along with a battery-powered light serving as a visual cue. The upper, accessible compartment contained either a reward (for correct choices) or no reward (for incorrect choices). Additionally, there was a lid that was magnetized, so it would remain in an upward state after the pig opened it.

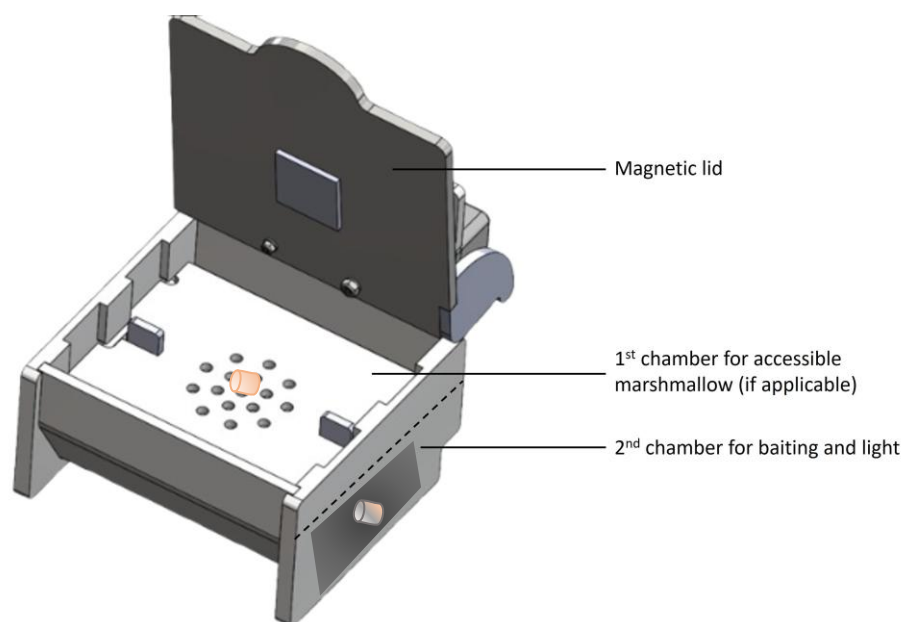


Figure 2.3 A diagram of the phases of the 8-arm radial maze (Hulbert and ShapeMaster, Ogden, IL, USA) conducted in a study assessing the cognitive differences between obese and lean Ossabaw pigs (n = 32; gilts and boars; Corvus Biomedical, LLC, Crawfordsville, IN).



Figure 2.4 A diagram showing possible configurations used in the acquisition phase of the 8-arm radial maze (Hulbert and ShapeMaster, Odgen, IL, USA) for assessing cognition of Ossabaw Pigs. Each pig was randomly assigned a unique configuration of 2 correct pods out of 8 total; correct pods had an accessible and inaccessible mini-marshmallow; incorrect pods had only an inaccessible mini-marshmallow. Pods 4 and 8 were never used in configurations for symmetry purposes, as pod 8 was the dedicated pod for exiting and entering the maze.

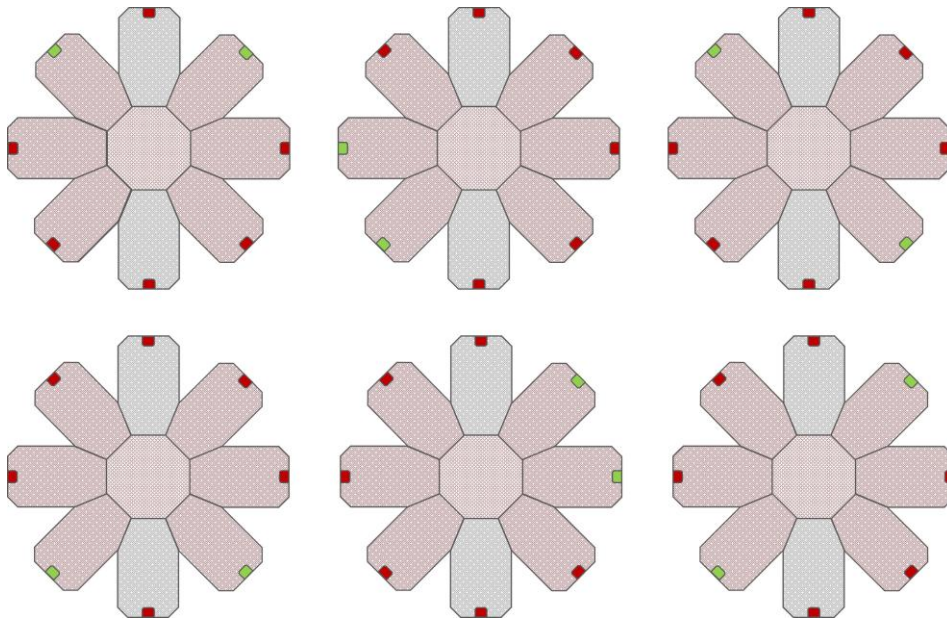


Figure 2.5 A diagram illustrating the threshold criteria used for observing and analyzing cognitive behaviors within the 8-arm radial maze (Hulbert and ShapeMaster, Ogden, IL, USA). The threshold allowed researchers to consistently determine when a pig had entered a specific area of the maze, supporting inter-observer agreement and accurate behavioral coding.

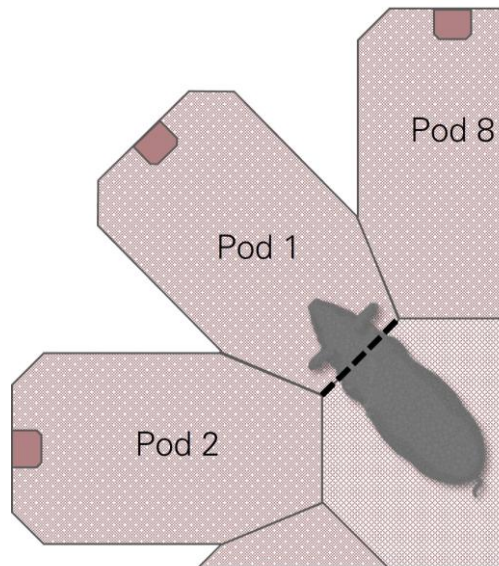


Figure 2.6 Conditioning phase completion measures of Ossabaw pigs (n=32, gilts and boars, pair housed) in the 8-Arm Radial Maze (Hulbert and ShapeMaster, Ogden, IL, USA). A. pod completion, % (i.e., entering all 8 pods; TRT*Week, $P = 0.001$). B. bowl completion, % (i.e., interacting with all 8 bowls) (TRT*Week, $P \geq 0.006$). (C) maze completion (i.e., entering all 8 pods and interacting with all 8 bowls) (TRT*Week, $P \geq 0.025$). Error bars represent standard error of the mean (SEM). Tukey-Kramer adjustment was used for pairwise comparisons.

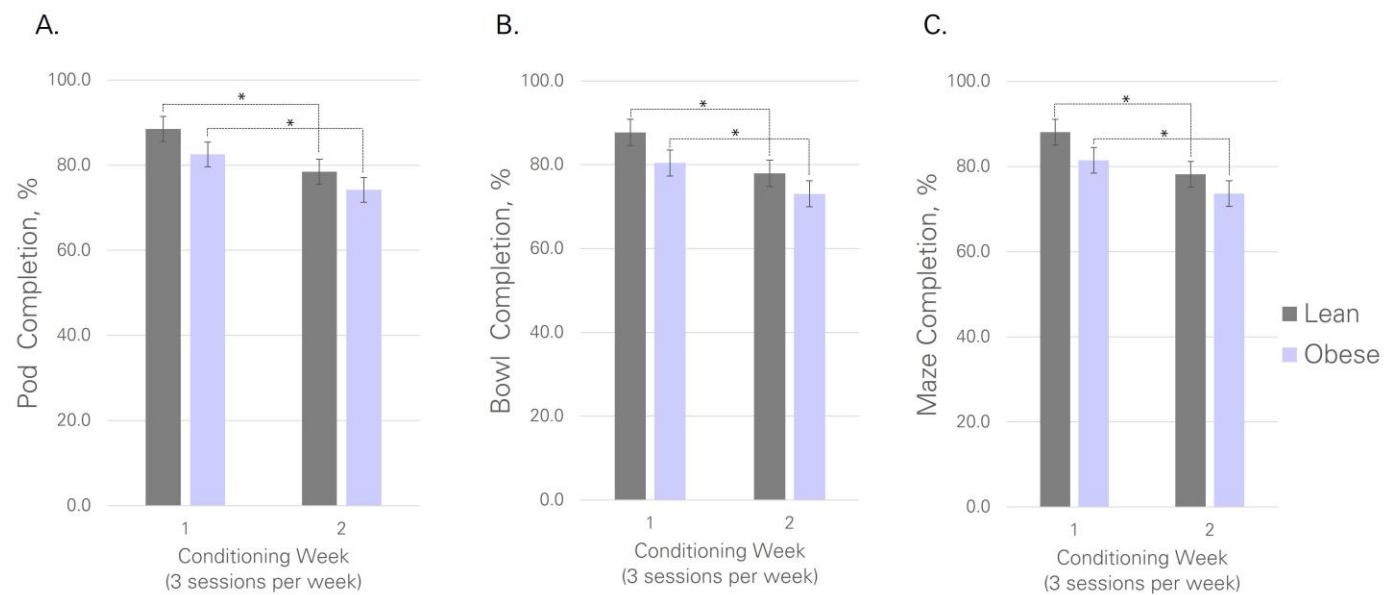


Figure 2.7 Conditioning phase latencies of Ossabaw pigs (n=32, gilts and boars, pair housed) in the 8-Arm Radial Maze (Hulbert and ShapeMaster, Ogden, IL, USA). A. Latency to last bowl in seconds (TRT*Week, $P \geq 0.003$). B. Latency to last pod in seconds (TRT*Week, $P \geq 0.038$). Error bars represent standard error of the mean (SEM), and Tukey-Kramer adjustment was used for pairwise comparisons.

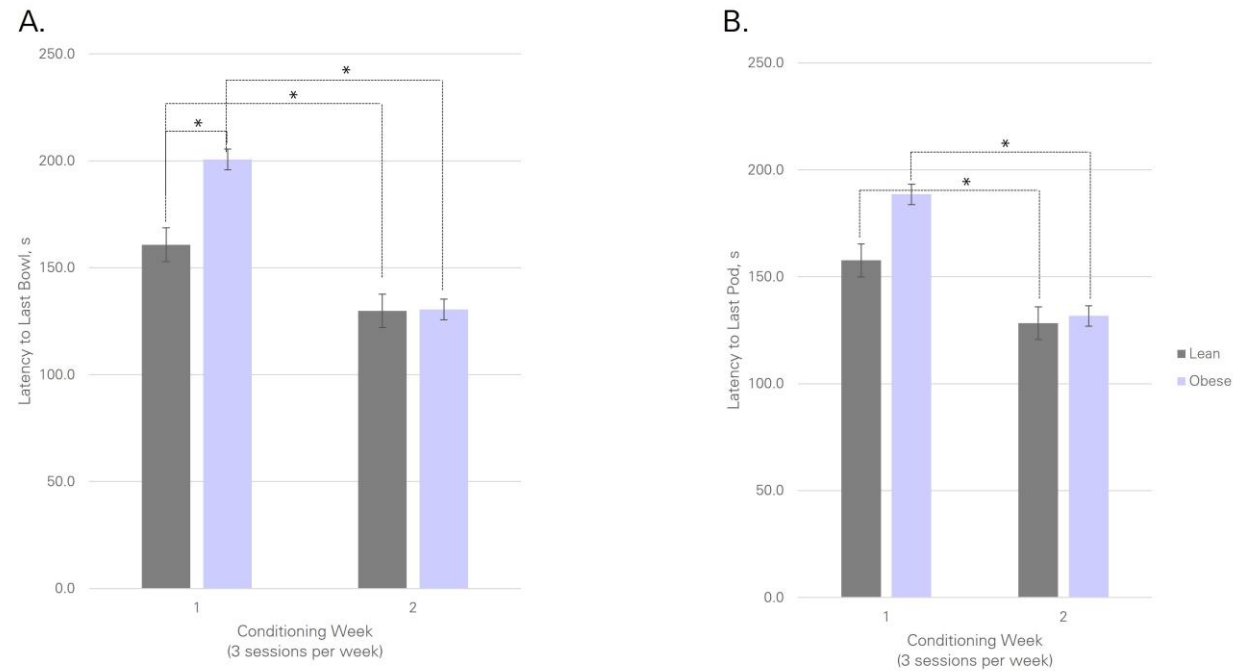


Figure 2.8 Conditioning phase durations of Ossabaw pigs (n=32, gilts and boars, pair housed) in the 8-Arm Radial Maze (Hulbert and ShapeMaster, Ogden, IL, USA). A. Maze duration, % (TRT*Week, $P \geq 0.005$). B. Bowl duration, % (TRT*Week, $P = 0.0001$). C. Pod duration (TRT*Week, $P = 0.0001$). Error bars represent standard error of the mean (SEM), and Tukey-Kramer adjustment was used for pairwise comparisons.

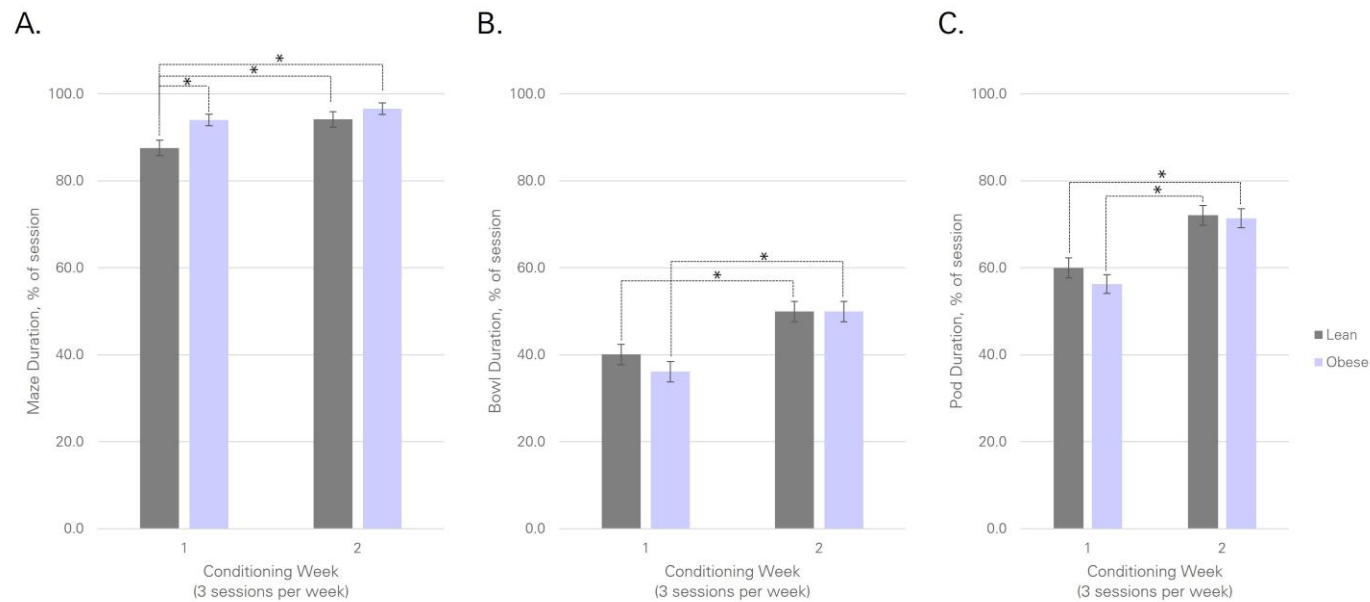


Figure 2.9 Conditioning phase entrance and center durations of Ossabaw pigs (n=32, gilts and boars, pair housed) in the 8-Arm Radial Maze (Hulbert and ShapeMaster, Ogden, IL, USA). A. Duration in entrance in seconds (TRT*Week, ≥ 0.005). B. Duration in center in (TRT*Week $P \geq 0.007$). Error bars represent standard error of the mean (SEM), and Tukey-Kramer adjustment was used for pairwise comparisons.

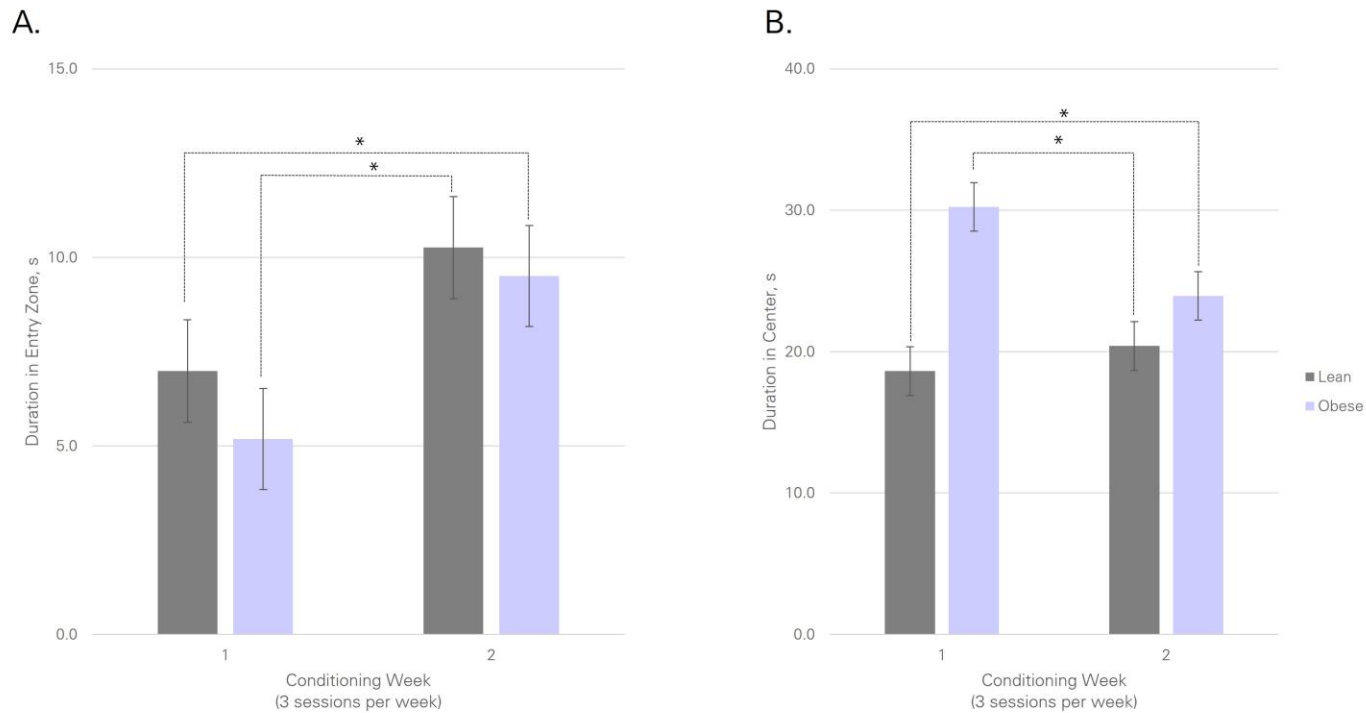


Figure 2.10 Acquisition phase center measures of Ossabaw pigs (n=32, gilts and boars, pair housed) in the 8-Arm Radial Maze (Hulbert and ShapeMaster, Ogden, IL, USA). A. latency to the center in frames/second (TRT*Week $P \leq 0.05$). B. duration spent in center in percent of session (TRT*Week, $P \leq 0.05$). Error bars represent standard error of the mean (SEM), and Tukey-Kramer adjustment was used for pairwise comparisons.

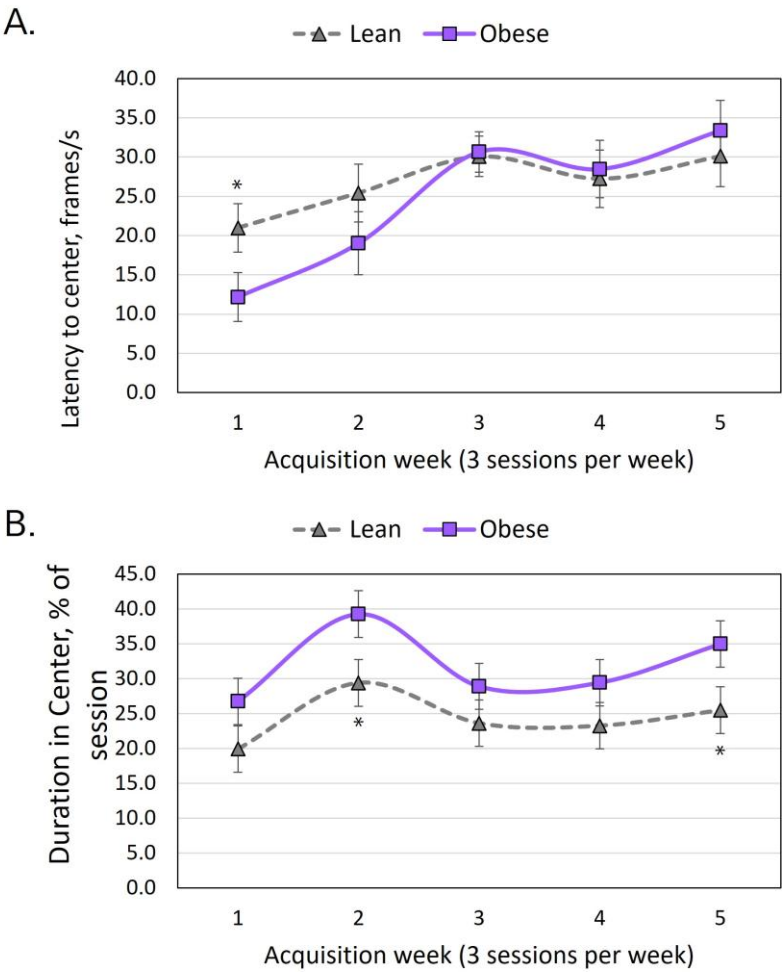


Figure 2.11 Acquisition phase latency measures of Ossabaw pigs (n=32, gilts and boars, pair housed) in the 8-Arm Radial Maze (Hulbert and ShapeMaster, Ogden, IL, USA). latency in seconds to first correct. (TRT*Week $P \leq 0.05$. B. latency in seconds to second correct pod (TRT*Week $P \leq 0.05$). C. latency in seconds to first correct (TRT*Week $P \leq 0.05$). D. latency in seconds to the second correct bowl (TRT * $P \leq 0.05$). Error bars represent standard error of the mean (SEM), and Tukey-Kramer adjustment was used for pairwise comparisons.

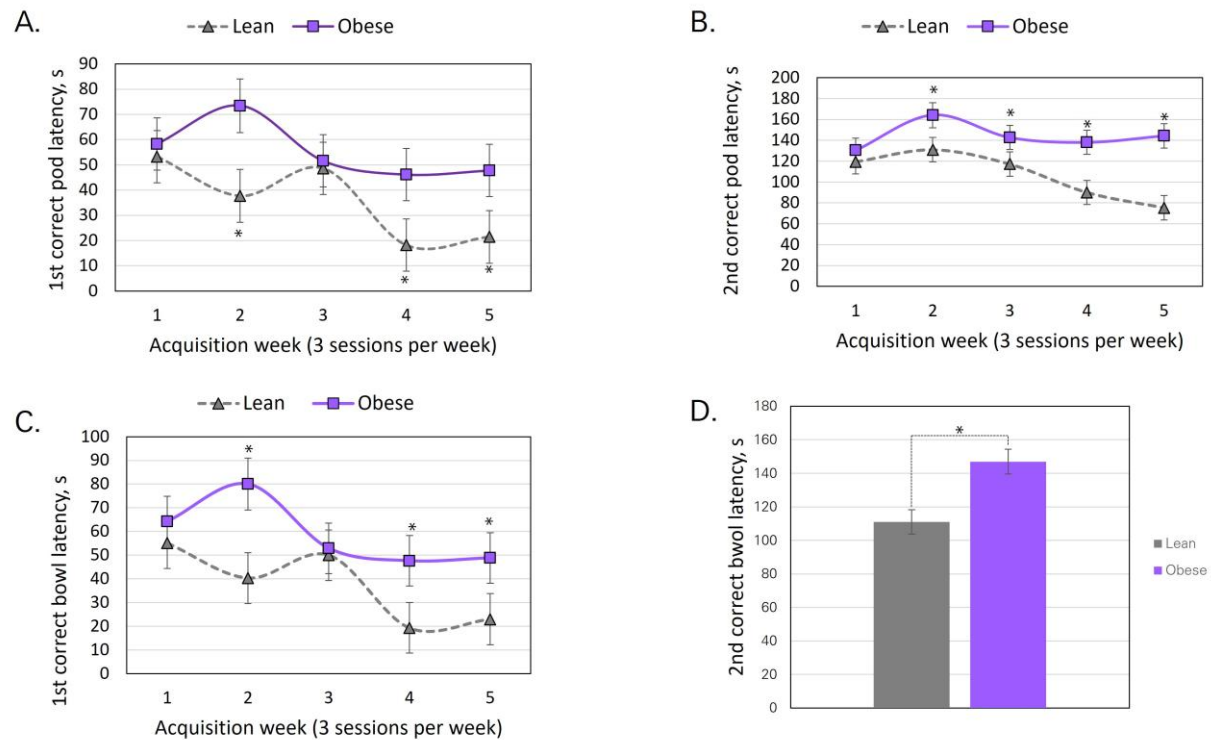
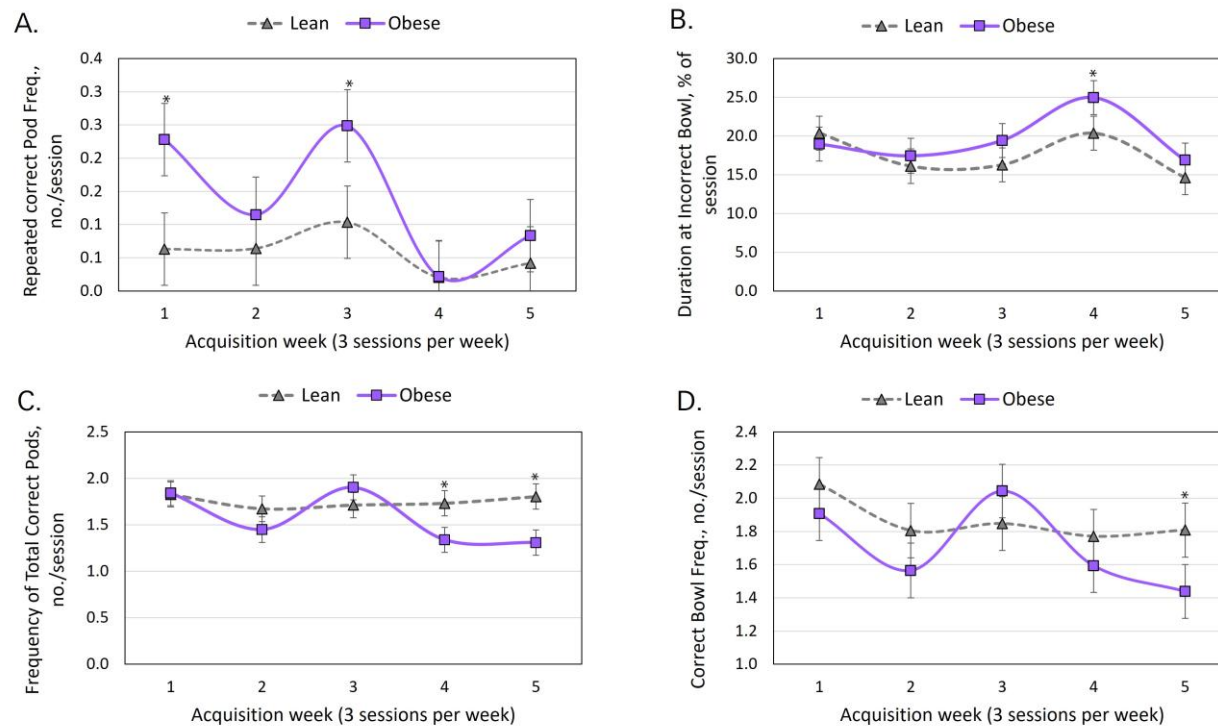


Figure 2.12 Acquisition phase frequencies and duration measures of Ossabaw pigs (n=32, gilts and boars, pair housed) in the 8-Arm Radial Maze (Hulbert and ShapeMaster, Ogden, IL, USA). A. repeated correct pod frequency (e.g., reentering a pod after already entering once before (TRT*Week $P \leq 0.05$). B. time spent at incorrect (TRT*Week $P \leq 0.05$). C. The number of times the pig entered correct pods (TRT*Week $P \leq 0.05$). D. The number of time pigs checked correct bowls (TRT*Week $P \leq 0.05$). Error bars represent standard error of the mean (SEM), and Tukey-Kramer adjustment was used for pairwise comparisons.



Chapter 3 - Blood Biomarkers in Obese vs. Lean Ossabaw Gilts and

Boars

Abstract

This study aimed to investigate the relationship between early-stage obesity and blood biomarkers in Ossabaw pigs (CorVus, Crawfordsville, IN; $n = 32$; boars and gilts; diets applied at 3.3 ± 0.52 months of age) fed a Western Diet for 16 weeks, with samples collected just before diets were applied (baseline, covariate) and at weeks 6 and 16 relative to starting the diet.

Hemoglobin tended to be higher in all obese pigs at week 16 ($P = 0.057$), and platelet counts tended to be higher overall ($P = 0.061$). Platelet concentrations were greater in boars than in gilts ($P = 0.035$). Overall, leukocyte counts did not differ ($P \geq 0.05$) between treatments or between boars and gilts but were within healthy ranges. Nonetheless, there were some differences in the leukocyte differentials. Compared to their lean counterparts, obese pigs had $4.8 \pm 1.35\%$ SEM less neutrophils ($P = 0.018$) and $5.3 \pm 1.31\%$ SEM more lymphocytes ($P = 0.008$), which resulted in a lower neutrophil-to-lymphocyte (NL) ratio ($P = 0.010$). In addition, lean pigs had X times more eosinophils than obese pigs ($P = 0.047$). Lymphocyte percentage was $7.9 \pm 1.29\%$ SEM greater in gilts than in boars ($P < 0.001$). Neutrophil percentage was greater in boars than gilts ($P < 0.001$; 31.9 vs $23.9 \pm 1.36\%$ SEM), and the NL ratio was also greater in boars than gilts ($P < 0.001$; 0.54 vs 0.36 ± 0.032). Obese pigs had higher BUN overall ($P < 0.001$). BUN concentrations were greater in gilts than boars ($P = 0.020$; 12.7 vs 9.8 ± 0.72 units, SEM), as well as the BUN: Creatinine ratio ($P = 0.004$; 10.4 vs 8.3 ± 0.55), total bilirubin ($P = 0.016$; 0.388 vs 0.278 ± 0.3126 SEM), and cholesterol ($P < 0.005$; 293.2 vs 189.8 ± 18.92 units SEM). Phosphorus was greater in obese pigs ($P < 0.001$). Albumin was greater in obese pigs at 6 weeks but lower than in lean pigs by 16 weeks (TRT*Time $P = 0.001$), and the albumin-to-globulin

ratio was higher in lean pigs at 16 weeks (TRT*Time $P = 0.026$). Alkaline phosphatase was elevated in obese pigs ($P = 0.001$), and GGT shifted over time, being higher in lean pigs at 6 weeks and higher in obese pigs by 16 weeks (TRT*Time $P = 0.037$). Total bilirubin was greater in obese pigs at both time points but declined over time in both groups (TRT*Time $P = 0.001$). Cholesterol was higher in obese pigs overall ($P = 0.040$). Amylase was higher in obese pigs at 6 weeks but higher in lean pigs at 16 weeks (TRT*Time $P = 0.026$). Lipase was higher overall in obese pigs (TRT*Time $P = 0.028$). These results demonstrate that early-stage obesity in pigs leads to measurable physiological changes, including shifts in lipid metabolism, inflammation, and hematology. Further studies need to include the lipid metabolism in gilts vs. boars, especially as steroid hormones may play a significant role.

Keywords: Obesity; Obese; Biomarkers; Complete Blood Count; Serum Chemistry; Ossabaw; Porcine; Swine; Pig; Inflammation

Introduction

Obesity is recognized as a chronic disease characterized by an overaccumulation of fat stores in adipocytes and is frequently associated with inflammation in adipose tissue and insulin resistance in peripheral tissues (Haslam and James, 2005; Spurlock and Gabler, 2008; Hernandez et al., 2017). Obesity commonly predisposes individuals to various metabolic disorders, primarily as a consequence of a persistent pro-inflammatory state (Spurlock and Gabler, 2008; O'Neill and O'Driscoll, 2015; Zhou et al., 2019). Research efforts are often aimed at understanding the biomarkers of obesity and its comorbidities in humans, frequently utilizing animal models such as rats and, more recently, pigs (Spurlock and Gabler, 2008). Humans are not the only species that are at risk of overnutrition. Commercially raised pigs, especially pregnant sows, are also at risk.

Inflammation-driven pathology can have significant long-term effects on both the sow and her offspring (Heerwagen et al., 2010; Newell-Fugate et al., 2014). Perturbations to the intra-uterine fetal environment lead to permanent post-natal changes in the metabolism and health of the offspring, with these effects being carried over to the next generation by epigenetic changes (Heerwagen et al., 2010). Maternal overnutrition was reported to disturb fetal development, predisposing piglets to early development of metabolic syndrome, mitochondrial dysfunction, and diminished antioxidative capacity (Tian et al., 2019). Obese sows are prone to increased stillbirth rates (Oliviero et al., 2010; Cools et al., 2014; Tian et al., 2019), impaired colostrum and milk yield (Decaluwé et al., 2013), and decreased neonatal survival (Cerisuelo et al., 2008). While most swine-related obesity research has been done on pregnant sows and their offspring, it can impact all pigs in the commercial setting. The amount of fat deposition not only affects growth rate and feed conversion efficiency but also influences lean meat rate and pork quality (Zhang et al., 2019).

Hematologic and biochemical parameters are of significant importance for clinicians and researchers when assessing the health status of animals and humans. Accurate hematological and biochemical parameters and intervals are widely regarded as valuable standards for indicating disease diagnosis, growth performance, or nutritional condition in pigs, in addition to aiding clinicians and researchers in interpreting the results of tests on blood samples (Lumsden and Mullen, 1978; Friendship et al., 1984; Klem et al., 2010; Zhang et al., 2022).

Clinicians often use body mass index (BMI) to define obesity, although, it has notable limitations: it does not differentiate between fat and lean mass, nor does it reflect visceral adiposity, which is more strongly linked to metabolic and cardiovascular risks (Frühbeck et al., 2019; Neeland et al., 2019). Therefore, alternative measures such as waist circumference and

waist-to-hip ratio are often recommended for more accurate assessment of obesity-related health risks (WHO, 2011). In addition to these measures, specific blood-based biomarkers may provide further insight into the metabolic burden of obesity, offering a more comprehensive approach to risk stratification and early detection. Routine bloodwork, while essential for monitoring pig health, is often seen as expensive, labor-intensive, and brings up possible welfare concerns in the industry, making it less accessible for regular use (Perri et al., 2017).

The Ossabaw pig exhibits unique metabolic characteristics that make it an intriguing animal model for studying obesity in humans (O'Neill and O'Driscoll, 2015; Sturek et al., 2020) and has been considered the most appropriate and promising model for metabolic research (Cluzel et al., 2022). The Ossabaw pig has a “thrifty genotype” that is believed to have conferred a selective advantage to populations in environments with unpredictable and fluctuating food supplies (Brisbin and Mayer, 2001; Lloyd et al., 2006). This genetic trait would have helped the pigs survive during periods of food scarcity, allowing them to efficiently store energy during times of abundance and endure starvation during lean periods (Sturek et al., 2007; O'Neill and O'Driscoll, 2015; Sturek et al., 2020). By using the Ossabaw pig, which is known for its propensity to develop obesity and related metabolic conditions (Clark et al., 2011; Newell-Fugate et al., 2014; Cluzel et al., 2022; Eickelmann et al., 2024), we can gain valuable insights into the hematological and biochemical effects of obesity. Given the similarities between human and pig physiology (Dawson, 2011; Merrifield et al., 2011; Roura et al., 2016; Lunney et al., 2021), especially in terms of metabolic processes, cardiovascular health, and organ function, the Ossabaw pig serves as an excellent translational model. This allows us to improve our knowledge of commercial pigs, but also how these findings could potentially impact human health.

Due to the Ossabaw pig's valuable role in translational research, blood samples are typically collected solely to meet specific study objectives. As a result, there is a lack of baseline data available for healthy Ossabaw pigs, including both boars and gilts. Oftentimes, baseline values from Yucatan pigs are used for comparison due to the similarities between the breeds (Dyson et al., 2006; Neeb et al., 2010). However, blood values are both species- and breed-dependent, making it essential to establish a stronger foundation of baseline parameters for accurately assessing the health of Ossabaw pigs. Notably, studies have shown differences in blood work, particularly in response to high-fat, high-calorie diets (Neeb et al., 2010). Existing data are available from trapped wild and domestically raised adult male Ossabaw pigs (Sturek, 2016), but this further emphasizes the gap in research by excluding gilts and sows. Kleinbongard et al. (2022) collected blood parameters from both gilts and castrated males, but there was no control group, as all pigs underwent induced blood flow restriction. Kreutz et al. (2011) measured baseline bloodwork was obtained from Ossabaw pigs on a lean, healthy, but sex was not reported, leaving ambiguity about the applicability of the results across sexes. Sham et al. (2014) used only female Ossabaw pigs and obtained baseline blood parameters from healthy, non-treated individuals, making it one of the few studies to do so.

Across studies using a full Western Diet in Ossabaw pigs, consistent metabolic disturbances were observed, including hyperglycemia, dyslipidemia, and liver-related changes, though biomarker data were often lacking. Importantly, there is notable variation in sex, age at diet initiation, and duration of dietary exposure, which may influence the severity and progression of MetS-related outcomes.

The objective of this study is to investigate the relationship between pre-pathologic obesity and biomarkers in Ossabaw pigs, after being on a Western Diet for 16 weeks, with

samples being obtained at both the 6-week mark and the 16-week mark. Additional objectives are to evaluate biomarker differences between sexes, as well as expand on current research on the baseline biomarkers provided for Ossabaw pigs (including boars and gilts), as well as handling during sample collection. We hypothesized that biomarkers of Ossabaw pigs fed a Western Diet would differ from Ossabaw pigs fed a standard, lean chow.

Materials and Methods

All experimental procedures adhered to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (IACUC #4831). All procedures were reviewed and approved by the Kansas State University's Ethics Committee for additional oversight.

Animals, housing, and husbandry

Ossabaw pigs (n = 32; 16 gilts and 16 boars, starting at age 15 weeks; Corvus Biomedical, LLC, Crawfordsville, IN) were used in two blocks (16 pigs per block), with each block consisting of pairs matched by body weight and sex. Sample size calculation was completed similarly to Nelsen et al., (2023), where the calculation was completed using variables from veterinary epidemiology methods previously described (Martin et al., 1987). The calculator was set to an alpha of 0.05 and the power (1-beta) to 95. Each measure's standard error was entered, and the desired magnitude of difference between sexes for each measure was entered.

Each pair was randomly assigned (3.3 ± 0.52 months of age) a diet formulated to maintain leanness (LabDiet Mini-Pig HF Grower 5L80; Purina Test Diet, Richmond, IN) or a western diet (KT324; Corvus Biomedical, Crawfordsville, IN). Prior to the Western Diet and control lean diet being applied, all pigs were fed a standard grower chow (DuMOR Hog Grower; Purina, Richmond, IN). The lean diet (up to 1,650 KCal per day; LabDiet 5L80, Purina, MO) supports healthy growth and leanness, consisting of 9.9% fat (measured as ether extract), 70.3%

carbohydrates, and 19.7% protein. In contrast, the western diet (KT324, CorVus Biomedical, IN) is a hypercaloric (up to 3,400 KCal Per day), high-fat, and high-fructose diet designed to induce obesity, with 40.8% of its carbohydrate-derived calories coming from high-fructose corn syrup, 42.9% of its fat-derived calories from sources such as ether extract, and a protein content of 16.9%. To minimize digestive upset, pigs assigned to the Western diet underwent a 9-day step-up period in which increasing proportions of the Western diet were gradually integrated into their original lean diet. In the first block, the western diet was given in a mash form, which caused complications with the automated feeding system, so in the second block, the western diet was given in pellet form. At age 23 weeks, pigs were transported for 8 h to Kansas State University's Large Animal Research Center (LARC). At LARC, pigs were housed in a climate-controlled facility. Pens were 3.7 x 3.7 m and were fitted with a feeding station that was 1.6 x 1.2 m. Pigs received water ad libitum, were fed twice daily, and given multiple types of environmental enrichment, cycled in and out of pens on a schedule throughout the study, such as Astros (EasyFix; SD, USA), Lunas (EasyFix; SD, USA), fixed bells, and milk jugs. In the 2023 block, pigs were provided water via a nipple drinker system, whereas in Block 2, lixer valves were used in conjunction with cast iron bowls (SMB Manufacturing, Wallenstein, Ontario).

Blood Sample Collection

Baseline blood samples were collected at 15 weeks of age before the test diets were introduced, and again after 6 weeks on the test diets (21 weeks of age), and after 16 weeks on the test diets (31 weeks of age). In the first block, collection dates were May 01, 2023, June 21, 2023, and August 29, 2023, respectively. In the second block, the collection dates were February 1, 2024, March 22, 2024, and May 28, 2024, respectively. The first two blood samples were collected at the pigs' colony (Corvus Biomedical, Crawfordsville, IN), and the third was

collected onsite at LARC with similar methodology for collecting samples at both locations. Procedures were conducted early in the morning, ensuring that pigs were in a fasted state and could recover to receive their morning feeding close to the scheduled time.

Approximately one week after pigs arrived at LARC, trained technicians began acclimating the pigs to the Panepinto sling, formerly introduced as a humane, minimal-stress method to handle swine in the 1960s (Panepinto et al., 1983). The sling was also used at Corvus Biomedical to provide training even before the pigs arrived at the testing facility. One pig at a time, in a consistent order, students would guide the pig from their home pen to the sling area using a hog board and allow them to sniff and see the sling prior to gently guiding them onto the sling, in slow movement. Two trained students on either side of the sling used a hog board to create a funnel-like pathway into the sling, ensuring the pig would not walk to the left or right of the sling. Once the pig's limbs were positioned in the limb cut-outs, a reward was given. Fruity-flavored, mini marshmallows were used as rewards and were given only when the pig was calm. Once the pig was calm and a reward was given, a student used the hand crank and raised the sling. Again, a reward was given once the pig was calm. Students would then allow the pig time to relax, while students gently made contact with the pig by petting it. The pig would then be lowered and moved back to its home pen using hog boards. This process was repeated up to twice per week, at Corvus Biomedical and LARC.

Before venipuncture procedures, all necessary personnel, utensils, and equipment were prepared and ready to create an efficient flow of collection and to minimize the stress of the pigs. Using similar methods as the acclimation period, trained students used hog-boards to guide the pigs to the sling area, one at a time and in the same order. Once the pig was restrained in the sling, two licensed veterinarians applied an anesthetic mask connected it to an anesthetic

vaporizer. The veterinarians then used isoflurane at 1-5% as the anesthetic drug with the appropriate amount of oxygen, using a Vetamac anesthesia machine (Vetamac, Rossville, IN). Once the pig was fully anesthetized, the pig was positioned onto its back into dorsal recumbency. From there, the veterinarians applied an eye lubrication gel (Optixcare; Aventix; ON, Canada) and began venipuncture. An 18-gauge, 1.5-inch needle pre-coated with heparin for anti-clotting was used for blood collection. The needle was inserted into the cranial vena cava, and 12 mL of blood was drawn using 4–5 BD Vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ). Vacutainer tubes used for blood collection included anticoagulants EDTA and Heparin. As each tube was collected, a designated technician gently inverted the tubes to prevent clotting. Immediately after blood collection, the anesthetic mask was removed to minimize the pig's time under anesthesia. Upon waking, technicians repositioned the pig into ventral recumbency, allowing the sling to be lowered so the pig could walk out independently with minimal human assistance. The pig was then guided back to its home pen using two hog boards and given time to rest before feeding. In addition, a glucose meter (Contour Next, Basel, Switzerland) was also used with blood extracted from the Vacutainers using a 3 mL syringe immediately after collection.

Commented [EM1]: Were they anesthetized at Corvus?

Sample Analysis Preparation

Heparin and EDTA Vacutainers were immediately placed at 4°C, while serum samples were stored at room temperature until all samples were collected. One EDTA sample per pig was placed on a tube rocker until they reached room temperature, then hematology measures were collected (ProCytte, Idexx Westbrook, ME). Heparin samples were held at 4°C until chemistry panel analyses (Catalyst One, Idexx). Plasma (Heparin) was harvested after centrifugation (

644G, 25 minutes, 4°C) and stored at -20° for chemistry panels that needed to be quality checked at a later time.

Statistical Analysis

All statistical analyses for serum chemistry and CBC panels were conducted using SAS software (version 9.4, SAS Institute Inc., Cary, NC). Data were first checked for normality using the Shapiro-Wilk test in the UNIVARIATE procedure. If normality assumptions were violated, data transformations including logarithmic, root, inverse, or arcsine transformations were applied. If assumptions remained unmet, non-parametric alternatives such as the Kruskal-Wallis test were used. For repeated measures analyses, the following covariance structures were considered: first-order autoregressive [AR(1)], heterogeneous autoregressive [ARH(1)], first-order ante-dependence [ANTE(1)], and compound symmetry (CS). The best-fitting covariance structure was selected using the corrected Akaike's information criterion (AICC). The subject effect was defined as Pen (pig ID), with two pigs housed per pen. To account for within-pen correlation and repeated measurements across time, residual maximum likelihood (REML) estimation was used. The Satterthwaite approximation was applied to calculate degrees of freedom, accounting for pen-to-pen variation. The model included treatment (TRT), block, time, and their interactions as fixed effects. Sex was included as a covariate, and pig ID nested within pen was specified as a random effect. The session nested within the week was treated as a repeated measure. Least square means (LSMeans) with Tukey's adjustment were used for multiple comparisons. Non-significant individual fixed effects were removed from the model to improve model fit. A treatment difference of $P \leq 0.05$ was considered significant, and tendencies were considered if the P -value was between 0.05 and 0.10 after biological relevance was considered.

Results

Hemoglobin concentration tended (TRT*Time, $P = 0.057$; Table 3.1) to be higher in obese pigs at week 16 relative to starting the diet. Platelets tended (TRT $P = 0.061$; Figure 3.1; Table 3.1) to be higher in obese pigs overall. Neutrophil count was lower overall in obese pigs in comparison to lean pigs (TRT $P = 0.010$; Figure 3.1; Table 3.1), as well as neutrophil percentage (TRT $P = 0.018$; Table 3.1; Figure 3.1), eosinophil percentage (TRT $P = 0.047$; Figure 3.1; Table 3.1), and the neutrophil-to-lymphocyte ratio (TRT $P = 0.010$; Figure 3.1; Table 3.1). Lymphocyte percentage was higher overall in obese pigs (TRT $P = 0.008$; Figure 3.1; Table 3.1).

Blood urea nitrogen (BUN) was higher in obese pigs than lean pigs overall (TRT $P < 0.001$; Table 3.2) and tended (TRT*Time $P = 0.054$; Table 3.2; Figure 3.2) to be different across time points. The ratio of BUN-to-creatinine was greater in lean pigs at both time points (TRT*Time $P = 0.004$; Figure 3.2; Table 3.2). Phosphorus concentration was greater in obese pigs than lean pigs overall (TRT $P < 0.001$; Table 3.2).

Albumin concentration was greater in obese pigs at 6 weeks relative to starting the diet but then lower in comparison to lean pigs at week 16 relative to starting the diet (TRT*Time $P = 0.001$; Figure 3.2; Table 3.2). The albumin-to-globulin ratio was greater in lean pigs than in obese pigs at week 16 relative to starting the diet (TRT*Time $P = 0.026$; Figure 3.2; Table 3.2).

Alkaline phosphatase concentrations overall were greater in obese pigs (TRT $P = 0.001$; Table 3.2). Gamma glutamyl transferase concentrations were greater in lean pigs than in obese pigs at 6 weeks relative to starting the diet, and then less than in obese pigs at 16 weeks relative to starting the diet (TRT*Time $P = 0.037$; Figure 3.2; Table 3.2). Total bilirubin concentration was greater in obese pigs than lean pigs at both time points but decreased for both treatments over time (TRT*Time $P = 0.001$; Figure 3.2; Table 3.2).

Cholesterol concentration was greater in obese pigs at both time points, with lean pigs slightly increasing between time points (TRT*Time $P = 0.040$; Figure 3.2; Table 3.2).

Amylase concentration was greater in obese pigs than lean pigs at 6 weeks relative to starting the diet, but then at 16 weeks, it was greater in lean pigs (TRT*Time $P = 0.026$; Figure 3.2; Table 3.2). Lipase was greater in obese pigs overall in comparison to lean pigs (TRT $P = 0.028$; Table 3.2).

Platelet concentrations were greater in boars than gilts ($P = 0.035$; 350.7 vs 317.2 ± 10.71 K/uL). Lymphocyte percentage was greater in gilts than boars ($P < 0.001$; 69.3 vs $61.4 \pm 1.29\%$), as well as lymphocyte count ($P < 0.001$; 9.6 vs 8.1 ± 0.43 K/uL). Neutrophil percentage was greater in boars than in gilts ($P < 0.001$; 31.9 vs $23.9 \pm 1.36\%$). The NL ratio was also greater in boars than in gilts ($P < 0.001$; 0.54 vs 0.36 ± 0.032).

BUN concentrations were greater in gilts than in boars ($P = 0.020$; 12.7 vs 9.8 ± 0.72 mg/dL), as well as the BUN: Creatinine ratio ($P = 0.004$; 10.4 vs 8.3 ± 0.55), total bilirubin concentrations ($P = 0.016$; 0.388 vs 0.278 ± 0.3126 mg/dL), and cholesterol concentrations ($P < 0.005$; 293.2 vs 189.8 ± 18.92 mg/dL).

Discussion

This study examined how diet-induced obesity alters hematological and biochemical profiles in pigs over time, with blood samples collected at both 6 and 16 weeks on specialized diets. The results revealed clear physiological shifts that reflect both the metabolic burden of pre-pathological obesity and the progression of systemic changes over time. Several markers showed significant time effects regardless of body condition, while others highlighted distinct differences between lean and obese pigs or revealed interaction effects indicative of divergent trajectories across the study period.

Importantly, the pigs used in this study were still undergoing normal growth and development, which is known to influence hematological and biochemical values. For example, erythrocyte indices, leukocyte counts, kidney function markers, and liver enzymes can shift significantly between juvenile and adult stages. These age-related changes have been documented in multiple pig breeds, including pathogen-free Minnesota miniature pigs, where younger pigs showed lower red blood cells (RBC), hemoglobin, hematocrit, and protein concentrations, along with higher phosphorus and BUN concentrations (Yeom et al., 2012). While this study observed many of these same trends over time, the additional deviations seen in obese pigs suggest that obesity-induced physiological changes may diverge from normal developmental patterns. While this developmental baseline shift must be acknowledged, the additional differences observed between lean and obese pigs, particularly in inflammatory, metabolic, and renal indicators, suggest that obesity has a compounding effect on top of normal growth-related changes.

There is a gap in research as to how obesity impacts biomarkers of Ossabaw pigs, and to our knowledge, there is only one known publication with differences in hematology, pointing out an increase in platelet reactivity (Kreutz et al., 2011). There are also no known records of reference ranges for Ossabaw pigs.

Hemoglobin is a protein found in RBCs that carry oxygen throughout the body (Jensen et al., 1998). Pro-inflammatory cytokines secreted during obesity can impair the bone marrow's ability to produce RBCs (Ganz, 2006; González-Domínguez 2020) and might indicate why obese pigs in the current experiment had a slightly decreased concentration of hemoglobin at week 16 than at week 6. Platelets play a critical role in maintaining hemostatic balance and serve as a first line of defense when endothelial integrity is compromised. Beyond their role in clotting, they

contribute to inflammation, atherogenesis, and atherothrombosis by adhering to damaged blood vessels, accumulating at injury sites, and releasing bioactive mediators (Michelson, 2003). In the current study, obese pigs had slightly higher platelet counts in comparison to lean pigs, correlating with findings in another Ossabaw study (Kreutz et al., 2011), as elevated platelets are commonly associated with obesity and systemic inflammation (Michelson, 2003; Anfossi et al., 2009). Neutrophils are specialized phagocytic cells that evolved in vertebrates to orchestrate and carry out one of the body's most essential physiological defenses: the inflammatory response (Amulic et al., 2012). Lean pigs in the current study had higher neutrophil counts and percentages. In contrast, obese pigs had elevated lymphocyte percentages, surprisingly, leading to significantly lower NL ratios, a commonly used stress marker (El Brihi and Pathak, 2025). Neutrophils, when increased, can indicate infection, trauma, physical stress, inflammation, hepatitis, and lymphocytes, when high, can indicate infection, whether it be viral, bacterial, or parasitic (El Brihi and Pathak, 2025). Contrary to our hypothesis, neutrophil and lymphocyte concentrations, as well as the neutrophil-to-lymphocyte ratio, exhibited trends in the opposite direction than anticipated, which was unexpected. The NL ratio is commonly used in addition to cortisol as a marker for stress in both rats and pigs (Metz and Gonyou, 1990; Puppe et al., 1997; Hickman, 2017). Analyzing cortisol concentrations in future research may be important to determine whether the observed effect was due to stress exhibited in lean pigs.

Among the most notable findings were the significant alterations in markers of renal function. Blood urea nitrogen (BUN) is a clinical measure of the amount of nitrogen in the blood that comes from urea, a waste product formed in the liver during protein metabolism (Weiner et al, 2015). Creatinine is a waste product formed from the normal breakdown of creatine, a molecule involved in energy production in muscle and is filtered out by the blood of the kidneys

(Delanaye et al., 2017). Blood urea nitrogen and the BUN to creatinine ratio were lower in obese pigs, particularly at 6 weeks, suggesting possible differences in hepatic function or hydration levels (Gounden et al., 2025). Decreased concentrations of BUN in obese pigs might indicate that the animals have a lower rate of protein turnover in comparison with lean pigs, where similar findings occurred in a study using genetically obese (Ningxiang strain) pigs after 6.5 weeks on an obesity-inducing diet (He et al., 2012). Additionally, Ossabaw boars that were on an obesity-inducing diet for 20 weeks (Alloosh et al., 2008) had reduced concentration of BUN in comparison to their lean counterparts. In the current experiment, creatinine increased significantly over time in all pigs, but obese pigs maintained consistently lower BUN values. Despite differences observed, pigs of both treatments were within healthy reference ranges of 85 Minnesota mini pigs (i.e., boars and gilts; ages 1-36 months; Yeom et al., 2012).

The chemistry panel gave insight into protein metabolism. Albumin is the most abundant plasma protein produced by the liver that helps maintain oncotic pressure and acts as a carrier for hormones and fatty acids (Kajal and Pathania, 2021). Globulins are a diverse group of proteins including immunoglobulins, enzymes, transport proteins, and acute-phase proteins (Kajal and Pathania, 2021). Albumin concentrations and the albumin-to-globulin ratio declined with time, particularly in obese pigs, further supporting the presence of systemic inflammation, hepatic imbalance, or injury (Lala et al., 2025), but still within healthy reference ranges provided by Sturek (2016) using 7 Ossabaw boars, aged 12-15 months.

The chemistry panel further supported a metabolic shift, particularly in liver function. Alkaline phosphatase and GGT are both liver enzymes that play key roles in bile processing (Lala et al., 2025). Enzymes such as ALP were higher in obese pigs compared to lean pigs, and changes in GGT were also observed over time. While elevated GGT is typically associated with

obstructive liver disease, both groups showed a decrease in GGT concentrations over time; however, obese pigs maintained higher concentrations than lean pigs by week 16 relative to starting the diet. Alkaline phosphatase is also associated with obstructive liver diseases when elevated, but to a lesser extent (Lala et al., 2025). Elevated ALP concentrations have been found more commonly in human children and adolescents, due to increased osteoblastic activity (Lala et al., 2025). Similar findings for ALP and GGT were found in a study using Ossabaw gilts on an obesity-inducing diet after 16 weeks, in addition to decreased spontaneous pen activity (Panasevich et al., 2018). In the current study, bilirubin concentrations decreased in both treatments over time but remained elevated in obese pigs in comparison to lean pigs. All measures of liver function remained within healthy reference ranges provided by Sturek (2016) using 7 Ossabaw boars, aged 12-15 months.

Markers associated with lipid metabolism, heart health, and pancreatic function also revealed important trends. Cholesterol, its metabolites, and biosynthetic precursors are vital for maintaining cell membrane structure, absorbing dietary nutrients, regulating reproduction, responding to stress, and managing fluid, electrolyte, and calcium balance (Tabas, 2002). Cholesterol concentrations were significantly higher in obese pigs at both timepoints, similar to other studies using Ossabaw pigs on an obesity-inducing diet for various amounts of time (Dyson et al., 2006; Neeb et al., 2010; Kreutz et al., 2011; Newell-Fugate et al., 2014; Westover et al., 2016; Matthan et al., 2018; Panasevich et al., 2018), indicating that high cholesterol concentrations can be found as early as 6-16 weeks on the Western Diet. High cholesterol is commonly a precursor to the development of cardiovascular heart disease, commonly found in obese patients (Després et al., 2008). In the current study, cholesterol concentrations were well

above healthy reference ranges provided by Sturek (2016) using 7 Ossabaw boars, aged 12-15 months.

Two key pancreatic enzymes are amylase, which helps break down starches, and lipase, which aids in the digestion of fats (Harper, 2018). Amylase increased sharply by week 16 in all pigs, with lean pigs having a higher concentration by week 16 relative to starting the diet. Lipase concentrations were also higher in obese pigs, particularly at earlier time points, supporting early pancreatic involvement in metabolic adaptation. Elevated amylase and lipase concentrations might indicate acute pancreatitis, pancreatic trauma, acute cholecystitis, and hepatitis, which can all be associated with obesity (Wanless and Lentz, 1990; Jeong and Lee, 2012; Martinez et al., 2024). There is a gap in recent research on the possible relationship between typical aging in younger individuals and increased amylase, leading to the need for further research on why amylase might have increased in lean pigs. Both amylase and lipase concentrations in the current study were within healthy reference ranges of 85 Minnesota mini pigs (i.e., boars and gilts; ages 1-36 months), provided by Yeom et al. (2012).

Gilts exhibited higher cholesterol, bilirubin, lymphocytes, and BUN and BUN: Creatinine ratio, which may reflect differences in metabolic rate, liver function, or renal handling of nitrogenous waste. Across several studies, gilts have shown elevated cholesterol concentrations compared to boars (Kawaguchi et al., 2013; Kojima and Degawa, 2024), and a human-based review suggests that it may be due to females having a greater lipolytic sensitivity (Williams, 2004). The higher bilirubin concentrations observed in gilts may reflect sex-based differences in liver metabolism. While there is a lack of existing research on pigs, female rats have shown increased activity of bilirubin-processing enzymes, which can influence circulating concentrations and excretion (Muraca et al., 1983). While total lymphocyte counts are commonly

used to assess immune status, they may overlook important sex-based differences in immune cell subtypes. Studies in rodents have shown that females can exhibit significant shifts in T cell populations, even when total lymphocyte counts remain unchanged. This suggests that differences in lymphocyte profiles between boars and gilts may reflect underlying immunological variation not captured by total counts alone (Ridge et al., 2019). Sex-based differences in BUN concentrations may reflect variation in immune responses, as previous studies have shown that BUN concentrations increase more in gilts than in males during fasting, and can also vary with non-infectious immune stimulation, such as Poly (I:C) treatment (Camborough pigs; age 60 days; Rymut et al., 2021).

Boars showed higher neutrophil percentage and NL ratio and neutrophil percentage, potentially indicating greater basal stress or inflammatory tone from paired housing and therefore increased agonistic behaviors (Albrecht et al., 2012). Boars also showed greater concentrations of platelets, potentially indicating heightened inflammation (Anfossi et al., 2009). Similar results were found by Jayachandran et al. (2004), using age 5-6-month-old crossbred commercial pigs and was said to be due to an increase in gonadal hormones.

Conclusions

Together, these findings indicate that obesity, even in its early stages, induces measurable and progressive alterations in systemic physiology, closely paralleling changes observed in human obesity. By weeks 6 and 16 of the Western diet, clear differences emerged in lipid metabolism, inflammatory markers, and hematological profiles. Importantly, this study also contributes to the limited body of research examining sex-based differences in obesity-related blood biomarkers, highlighting the need for more inclusive datasets that consider both gilts and boars, as well as the lack of reference ranges. While routine blood chemistry and hematology

analyses may not always be feasible in commercial swine settings, employing low-stress handling methods such as the Panepinto sling can reduce sampling-related stress and improve the reliability of blood-based indicators. Collectively, these results support continued investigation into practical, early-detection biomarkers of obesity and stress in pigs, both for advancing biomedical understanding and enhancing welfare and management practices in the swine industry.

Implications

Obesity induces a range of physiological and hematological alterations in both humans and pigs, and the present findings demonstrate that such changes can emerge as early as six weeks into a Western-style diet. These early shifts highlight the urgency of developing timely dietary interventions. The evidence supports the need for nutritional strategies or feed additives that are not only effective in mitigating these immediate effects but also sustainable and accessible. Such interventions are especially important in populations facing food insecurity, where poor diet quality may accelerate metabolic dysfunction and compromise long-term health. Additionally, these findings underscore the potential for implementing similar dietary interventions within the swine industry to improve metabolic health, support animal welfare, and promote long-term productivity in over-conditioned or obese breeding animals.

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Tables

Table 3.1 Complete Blood Count panels of obese and lean Ossabaw Pigs (n=32, gilts and boars, pair housed) after 6 weeks and 16 weeks on a western diet, and standard grower chow, respectively.

	Lean	Obese	SEM	6 Weeks			16 Weeks			P- Values		
				Lean	Obese	SEM	Lean	Obese	SEM	Time	TRT	Time*TRT
Red Blood Cells, M/uL	8.1	8.0	0.16	7.8 ^a	8.0 ^a	0.19	8.4 ^b	8.1 ^b	0.19	0.051	0.786	0.146
Hemoglobin, g/dL	13.8	13.7	0.26	13.3 ^a	13.6 ^a	0.30	14.3 ^b	13.7 ^a	0.30	0.011	0.623	0.057
Hematocrit, %	47.1	47.9	1.00	47.6 ^a	50.0 ^a	1.21	46.6 ^b	45.9 ^b	1.21	0.014	0.586	0.118
MCV (fL)	58.7	59.3	0.71	61.3 ^a	62.2 ^a	0.84	56.1 ^b	56.5 ^b	0.84	0.001	0.547	0.702
MCH (pg)	17.2	16.9	0.13	17.2 ^a	16.9 ^a	0.15	17.3 ^a	16.9 ^a	0.15	0.472	0.094	0.795
Platelets, K/uL	319.2	348.7	10.71	302.2 ^a	341.4 ^a	14.03	336.2 ^a	356.1 ^a	14.03	0.067	0.061	0.457
White Blood Cells, K/uL	13.6	13.6	0.50	15.8 ^a	15.1 ^a	0.57	11.4 ^b	12.2 ^b	0.59	0.001	0.971	0.103
Lymphocytes, K/uL	8.6	9.2	0.43	9.2 ^a	9.6 ^a	0.46	8.0 ^b	8.9 ^a	0.47	0.001	0.297	0.379
Neutrophils, K/uL ¹	4.2	3.5	0.23	5.6 ^a	4.5 ^b	0.31	2.8 ^c	2.5 ^d	0.32	0.001	0.010	0.897
Monocytes, K/uL	0.56	0.55	0.035	0.65 ^a	0.61 ^a	0.056	0.48 ^b	0.49 ^b	0.037	0.004	0.763	0.620
Eosinophils, K/uL	0.39	0.33	0.030	0.42 ^a	0.34 ^a	0.033	0.35 ^b	0.31 ^b	0.034	0.026	0.184	0.443
Basophils, K/uL	0.01	0.02	0.001	0.02 ^a	0.02 ^b	0.002	0.01 ^c	0.01 ^c	0.002	0.001	0.090	0.205
Lymphocytes, %	62.7	68.0	1.31	57.8 ^a	63.6 ^b	1.84	67.5 ^c	72.5 ^d	1.26	0.001	0.008	0.760
Neutrophils, %	30.3	25.5	1.35	35.5 ^a	30.1 ^b	1.58	25.0 ^c	20.9 ^c	1.69	0.001	0.018	0.630
Monocytes, %	4.1	4.0	0.26	4.1 ^a	4.0 ^a	0.34	4.1 ^a	4.0 ^a	0.35	0.972	0.764	0.981
Eosinophils, %	2.8	2.4	0.16	2.5 ^a	2.2 ^a	0.19	3.2 ^b	2.5 ^c	0.19	0.001	0.047	0.243
Basophils, %	0.09	0.11	0.009	0.10 ^a	0.13 ^b	0.0114	0.08 ^b	0.08 ^b	0.012	0.003	0.121	0.139
NP Ratio ^{1,2}	0.52	0.40	0.036	0.65 ^a	0.49 ^b	0.0583	0.39 ^c	0.30 ^d	0.026	0.001	0.010	0.882

^{a,b,c,d} Using the Tukey-Kramer adjustment, LS Means differ ≤ 0.05 ; Some *P*-values were generated from ¹ Inverse transformed data; Raw data are presented with transformed SEM; ²Neutrophil-to-lymphocyte ratio.

Table 3.2 Chemistry panels of obese and lean Ossabaw Pigs (n=32, gilts and boars, pair housed) after 6 weeks and 16 weeks on a western diet, and standard grower chow, respectively.

	Lean	Obese	SEM	6 Weeks			16 Weeks			P- Values		
				Lean	Obese	SEM	Lean	Obese	SEM	Time	TRT	Time*TRT
Glucose, mg/dL	89.6	86.1	3.96	91.0 ^a	91.4 ^a	4.79	88.2 ^b	80.8 ^b	3.71 ^b	0.008	0.537	0.106
Creatinine, mg/dL	1.2	1.3	0.03	1.1 ^a	1.2 ^a	0.03	1.3 ^b	1.3 ^b	0.03	0.001	0.281	0.381
BUN, mg/dL ³	14.0	8.5	0.74	13.9 ^a	7.8 ^b	0.77	14.0 ^a	9.2 ^c	0.77	0.024	0.001	0.054
BUN: Creatinine Ratio	11.75	6.94	0.622	12.69 ^a	6.75 ^a	0.572	10.8 ^b	7.13 ^c	0.81	0.046	0.001	0.004
Phosphorus, mg/dL	6.9	8.3	0.14	7.2 ^a	8.8 ^b	0.16	6.6 ^c	7.8 ^d	0.16	0.001	0.001	0.165
Calcium, mg/dL ¹	7.5	7.6	0.04	5.4 ^a	5.4 ^a	0.08	9.6 ^b	9.8 ^b	0.08	0.001	0.051	0.185
Total Protein, g/dL	7.5	7.5	0.12	7.5 ^a	7.6 ^a	0.18	7.6 ^a	7.5 ^a	0.11	0.791	0.886	0.294
Albumin, g/dL	4.1	4.0	0.06	4.1 ^a	4.3 ^a	0.07	4.0 ^b	3.8 ^b	0.07	0.001	0.537	0.001
Globulin, g/dL	3.4	3.6	0.09	3.3 ^a	3.4 ^a	0.11	3.6 ^b	3.8 ^b	0.11	0.001	0.231	0.371
Albumin: Globulin Ratio	1.24	1.15	0.036	1.32 ^a	1.32 ^a	0.044	1.16 ^b	0.98 ^c	0.04	0.001	0.100	0.026
Alanine Transaminase, U/L	68.7	63.1	5.17	71.9 ^a	64.7 ^a	5.55	65.5 ^a	61.6 ^a	5.55	0.109	0.457	0.551
Alkaline phosphatase, U/L	103.3	159.9	10.25	62.8 ^a	110.6 ^b	13.05	143.9 ^c	209.3 ^c	13.05	0.001	0.001	0.445
GGT, U/L ²	52.5	51.9	3.22	59.4 ^a	53.1 ^a	3.71	45.6 ^b	50.7 ^b	3.71	0.004	0.897	0.037
Bilirubin, mg/dL	0.22	0.45	0.032	0.29 ^a	0.59 ^b	0.046	0.14 ^c	0.30 ^a	0.033	0.659	0.985	0.001
Cholesterol, mg/dL	101.3	381.7	19.48	96.3 ^a	388.8 ^b	18.53	106.3 ^a	374.6 ^b	21.16	0.717	0.001	0.040
Triglycerides, mg/dL	29.2	24.7	2.17	29.3 ^a	25.0 ^a	3.01	29.2 ^a	24.3 ^a	3.01	0.900	0.150	0.933
Amylase, U/L	601.4	601.2	28.92	445.0 ^a	494.6 ^a	32.58	757.8 ^b	707.8 ^b	32.58	0.001	0.996	0.026
Lipase, U/L	15.8	25.8	3.05	20.4 ^a	28.8 ^b	4.13	11.3 ^a	22.9 ^a	4.13	0.070	0.028	0.684
Fructosamine, umol/L	143.6	145.7	6.98	140.3 ^a	145.4 ^a	10.93	146.8 ^b	145.9 ^b	4.95	0.609	0.834	0.667

^{a,b,c,d} Using the Tukey-Kramer adjustment, LS Means differ ≤ 0.05 ; Some *P*-values were generated from ¹ Inverse transformed data; Raw data are presented with transformed SEM; ²Gamma-Glutamyl Transferase; ³Blood urea nitrogen.

Figures

Figure 3.1 Fold-change heatmap showing relative changes in complete blood count (CBC) markers between lean and obese pigs at 6 and 16 weeks on their respective diets. Colors represent the fold change in marker concentrations (Obese/Lean), with warmer tones indicating lower values in obese pigs and cooler tones indicating higher values. Statistical significance of Treatment x Time (*P < 0.05; Tukey-Kramer adjustment).

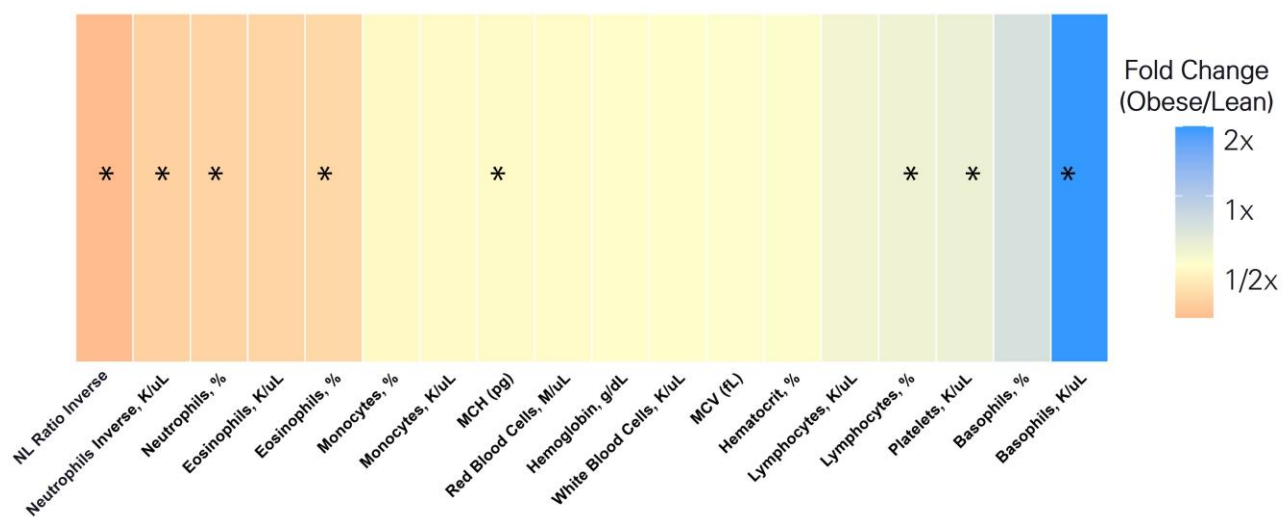
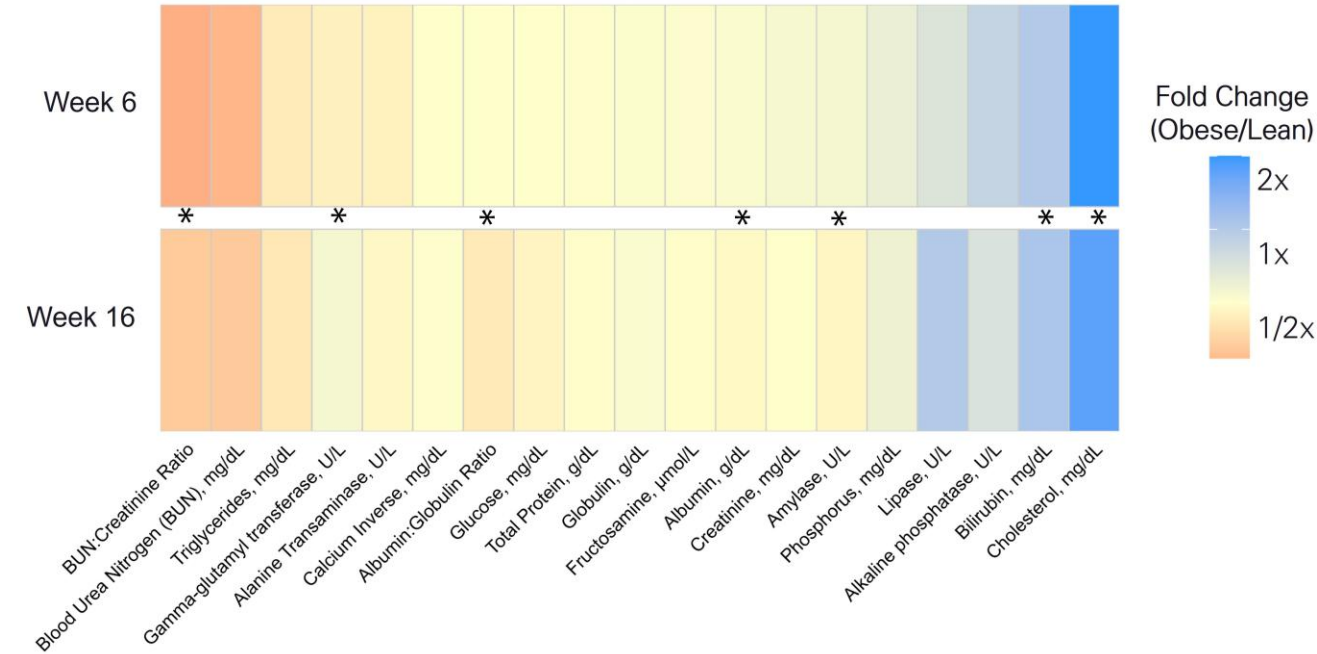


Figure 3.2 Fold-change heatmap showing relative changes in blood chemistry markers between lean and obese pigs at 6 and 16 weeks on their respective diets. Colors represent the fold change in marker concentrations (Obese/Lean), with warmer colors indicating lower values in obese pigs and cooler colors indicating higher values. . Statistical significance of Treatment x Time (*P < 0.05; Tukey-Kramer adjustment).



Chapter 4 - General Conclusions and Future Directions

Abstract

This final chapter serves as a synthesis of the work presented throughout the thesis, reflecting on the broader implications of the umbrella study investigating early-stage diet-induced obesity in Ossabaw pigs. The research aimed to assess both physiological and behavioral outcomes before overt metabolic disease, while emphasizing current efforts in exploring the potential of fermented sorghum tea (sorghum kombucha) as a functional dietary intervention. Emphasis is placed on the translational relevance of findings to both biomedical and agricultural contexts, particularly within the swine industry, where obesity presents challenges to reproduction, cognition, and overall welfare. This chapter also discusses future research directions, including analysis of spatial sequencing in cognitive tasks and continued investigation into sustainable feed additives. Collectively, this chapter underscores the need for proactive dietary strategies and provides a foundation for integrating nutritional interventions into obesity management frameworks for both animals and humans.

Keywords: Obesity; Obese; Ossabaw; Cognition; Biomarkers; Sorghum; Pig; Swine; Porcine

Umbrella Study

The work presented in this thesis aimed to investigate the physiological and behavioral outcomes of diet-induced obesity before the onset of overt metabolic pathology. This research is part of a broader effort to identify sustainable, functional food-based strategies that may mitigate the physiological consequences of obesity before the development of full-blown metabolic syndrome. Our lab is actively investigating the use of fermented sorghum tea, commonly known as sorghum kombucha, as a dietary intervention. Unlike many studies that focus on treating established disease, our approach targets a critical window prior to metabolic collapse,

intervening at a pre-pathological stage when the body is already showing signs of dysfunction but before irreversible damage occurs, where early dietary strategies like sorghum kombucha may have the greatest potential to preserve physiological function and prevent long-term complications.

Following 16 weeks on a high-fat, high-fructose Western diet, obese pigs were transitioned to the same standard diet consumed by lean pigs, mimicking real-world scenarios in which individuals adopt healthier eating habits in an effort to reverse weight gain and metabolic dysfunction. At this transition point, both obese and lean pigs were further divided into two groups: one receiving an isocaloric control solution (e.g., sugar water) and the other receiving fermented sorghum kombucha. In this design, sorghum kombucha serves not as a dietary replacement but as a targeted functional additive, allowing researchers to assess its potential to enhance metabolic recovery, reduce inflammation, and support physiological rebalancing during the early stages of dietary correction. This approach offers a translational model for how nutritional interventions might be integrated into post-obesogenic diets in both clinical and agricultural settings.

To evaluate the efficacy of this intervention, a comprehensive suite of physiological and anatomical assessments was conducted. Blood samples were collected both prior to and following the introduction of the sorghum tea to assess changes in metabolic, inflammatory, and oxidative stress biomarkers. Body composition was monitored using ultrasonography to measure backfat thickness and loineye area as indicators of adiposity and muscle distribution. Weekly body weights were recorded to track progression over time. Fecal samples were collected to support microbiome profiling and to assess gastrointestinal health, including potential dysbiosis

associated with both obesity and dietary shifts. At the study conclusion, pigs underwent full necropsy. Organ weights were recorded for major metabolic and immune organs, including liver, spleen, pancreas, kidneys, and brain, to evaluate structural adaptations to obesity and treatment. Additionally, a broad range of tissues was harvested and preserved for histological analysis, allowing for future investigation into cellular-level changes in response to the sorghum kombucha.

Together, this multifactorial approach enables a robust assessment of sorghum kombucha's potential to reduce inflammation, support metabolic restoration, and preserve tissue integrity in a model of pre-metabolic syndrome obesity. This study not only contributes to the growing body of agrimedical research but also offers translational insight into how dietary additives may enhance early-stage obesity management in both humans and animals.

Sorghum Kombucha

Sorghum (*Sorghum bicolor* L. Moench), a cereal in the Poaceae family native to Northeastern Africa, has been cultivated for up to 4,000 years (Rooney, 2000; Xiong et al., 2019). Ranked fifth in global cereal crop production behind maize, wheat, rice, and barley, sorghum is valued for its use in food, feed, fiber, and bioenergy (Wang et al., 2007; Xiong et al., 2019; Stefoska-Needham and Tapsell, 2020). In Asia and Africa, it serves as a key food source, particularly in underdeveloped regions, while in Western countries like the United States, Mexico, and Australia, it is primarily used for animal feed (Rooney, 2000; Wang et al., 2007; Xiong et al., 2019). Sorghum and maize are foundational in livestock diets, especially for swine, poultry, and cattle, where processing techniques improve palatability and nutrient efficiency (Rooney, 2000). More recently, sorghum's sustainability and climate resilience have driven its

emergence as a primary food crop in the U.S. (Rooney, 2000; Wang et al., 2016). Tolerant to drought, heat, and altitude, and requiring fewer inputs than maize, sorghum is well-positioned as a sustainable alternative under climate change (Xiong et al., 2019; Stefoska-Needham and Tapsell, 2020).

Nutritionally, sorghum is gluten-free, high in resistant starch, and rich in essential nutrients and phenolic compounds (Awika and Rooney, 2004; Xiong et al., 2019; Stefoska-Needham and Tapsell, 2020). Its phenolic profile varies widely by genotype, color, and growing conditions, and the grain is broadly classified into white, yellow, red, brown, and black varieties (Awika and Rooney, 2004; Xiong et al., 2019). The bran layer contains key phytochemicals, phenolic acids, flavonoids, tannins, sterols, and policosanols, which contribute to plant defense and offer antioxidant and cholesterol-lowering benefits (Dykes and Rooney, 2007; Xiong et al., 2019).

Oxidative stress, a result of excess reactive oxygen species, contributes to chronic diseases such as diabetes, atherosclerosis, and cardiovascular disorders (Xu et al., 2021). Sorghum's phenolic compounds, particularly condensed tannins, show strong antioxidant activity and potential for disease prevention, although most evidence to date comes from in vitro studies (Wolfe and Liu, 2007; Awika and Rooney, 2004; Xiong et al., 2019).

Chronic low-grade inflammation is a hallmark of obesity and is driven by elevated levels of cytokines like IL-1 β , TNF- α , and IL-6, as well as inflammatory mediators such as COX-2 and PGE2 (Xiong et al., 2019; Hong et al., 2020). Targeting these markers may reduce obesity-related comorbidities.

Obesity within the Swine Industry

While Ossabaw pigs are commonly used for translational studies in terms of obesity, metabolic disorders, and cardiac disease, we can also utilize Ossabaw pigs and the effects that obesity has on them and apply it to commercially raised pigs. The Ossabaw pig breed is predisposed to obesity and makes an excellent model to study the mechanism by which excessive fat deposition impairs sows' reproductive performance (Muro et al., 2023). According to a figure by Muro et al. (2023), obese sows demonstrate reduced productivity at multiple stages of their reproductive cycle. During rearing, they may experience increased musculoskeletal issues and decreased oocyte quality. In early pregnancy, obesity is associated with lower endometrial receptivity, an impaired uterine environment, reduced progesterone concentration, decreased embryo viability, and more significant variability in embryo size. As pregnancy progresses to the mid-to-late stages, obese sows may exhibit excessive fat accumulation in mammary tissue, increased oxidative stress in the placenta, abnormal placental lipid accumulation, and reduced placental vascularity and efficiency. During farrowing and lactation, they are more likely to experience prolonged labor, a higher risk of dystocia, increased body weight loss, reduced voluntary feed intake, and a greater risk of complications in subsequent pregnancies (Coffey et al., 1999; Muro et al., 2023).

We commonly see imbalanced nutrition in pregnant sows within the swine industry, which can lead to either undernutrition or obesity (Zhou et al., 2019). The link between energy balance and reproductive performance is critical, as producers want to ensure sows have the necessary nutrients for healthy pregnancies and strong piglets. Obesity in pregnant sows can lead to a variety of detrimental effects to themselves and their piglets, such as placental dysfunction,

altered hormone signaling, and systemic inflammation, causing decreased litter sizes (Li et al., 2019; Tian et al., 2019), lower piglet birth weight (Kim et al., 2015; Song et al., 2018; Li et al., 2019; Zhou et al., 2019), and fewer piglets born alive (Cools et al., 2014; Cheng et al., 2019). Obesity poses significant challenges beyond its immediate effects related to reproduction, as it can lead to structural and metabolic changes in multiple tissues and organs, such as muscle, uterus, and liver. In return, the effects on these organs can have a negative effect on reproduction and the health of the offspring (Fowden et al., 1995).

There is limited research on the impact obesity has on male pigs, but it is presumed that the male offspring of an obese sow are more susceptible to obesity. Rodent studies have found that obesity is correlated with reduced fertility, largely due to hormone imbalances linked to excess body fat (Mounzih et al., 1997; Teerds et al., 2011). Studies in mice and rats suggest that elevated levels of leptin and resistance to its effects can interfere with signals from the brain that control testosterone production and sperm development (Mounzih et al., 1997; Teerds et al., 2011). Some studies using Göttingen minipigs have shown that female pigs are more likely to become obese than male pigs as they gain weight faster (Bollen et al., 2005; Mentzel et al., 2015). Male minipigs have higher levels of testosterone and estradiol, which may offer some protection against fat accumulation. In contrast, females exhibit greater insulin resistance and higher leptin levels, contributing to increased fat storage and a higher risk of metabolic disturbances (Mentzel et al., 2015). Based on findings in this thesis, we can emphasize the need for dietary intervention or strict dietary protocols within the swine industry to prevent the negative physiological outcomes of obesity.

Cognition within the Swine Industry

As presented in this thesis, there may be a link between obesity and cognitive decline in Ossabaw pigs. Cognitive research enhances our understanding of their welfare and cognitive needs in industry (Held et al., 2001; Bushby et al., 2018). Cognition has been shown to affect livestock production both directly and indirectly. Indirect effects occur when stressors or a lack of a mentally stimulating environment cause developmental cognitive deficits, compromising welfare and negatively affecting productivity (Held et al., 2001). Direct effects happen when animals' cognitive abilities directly impact their productivity, such as failure to locate food and interacting socially without aggression (Held et al., 2001). Modern production systems rely on the ability of pigs to learn and remember how to use unfamiliar equipment and where to find important resources (Held et al., 2001). While cognitive tests aren't to be seen in commercial settings, utilizing them in the laboratory can provide insights into the cognitive needs of pigs in industry, and what might be worsening or improving cognitive ability (Held et al., 2001; Ede and Parsons, 2023). Most cognitive testing tasks serve as a measure of enrichment, while also being necessary for maintenance behavior, allowing researchers to understand how this enrichment affects livestock behaviors and how it might improve their overall well-being and productivity (Held et al., 2001; Puppe et al., 2007). At weaning in the commercial setting, piglets must learn how to acquire food from unfamiliar sources, requiring problem-solving skills and sufficient cognitive abilities. Problem-solving abilities in the commercial setting are also required in group-housed pigs, where social status plays a large role (Brajon et al., 2021). Social status affects access to resources; dominant pigs often secure better feeding opportunities, while subordinates may experience restricted access, impacting growth and health. Furthermore, pigs demonstrate

cognitive abilities, such as problem-solving and cooperation, which are influenced by their social environment (Brajon et al., 2021).

Sequencing of the 8-ARM

Future analysis may examine how pigs navigate the 8-Arm Radial Maze (8-ARM) using sequence-based strategies, which could provide deeper insights into their cognitive processes and efficiency under varying physiological states, such as obesity. Staddon (1983) introduced the concepts of temporal and spatial processes to distinguish between working and reference memory, respectively. These frameworks help classify maze behaviors as random choice, sequencing, true spatial mapping, or advanced strategies like chunking (Table 4.1; Staddon, 1983; Dallal and Meck, 1990).

A variety of strategies have been documented in rodents using the 8-ARM, such as the adjacent arm or "1" strategy, which involves moving sequentially from one arm to the next, and angular strategies like the "3" (135°) or "4-3" (180°) patterns, which may reflect attempts to reduce memory load through predictable movements (Foreman and Gillett, 2013). Apparatus design, motivation (e.g., water vs. food rewards), and prior experience can significantly influence which strategy is adopted (Foreman, 1985; Innis and Macgillivray, 1987; Okaichi and Oshima, 1990). While some strategies suggest more advanced spatial cognition, others may emerge as compensatory mechanisms in subjects with cognitive impairment (Figure 4.1) or increased cognitive demand (Winocur, 1982; Pico and Davis, 1984).

Identifying patterns of sequencing in pigs, particularly under different dietary or physiological conditions, could help determine how cognitive load is managed and whether obesity interferes with higher-order strategy use. This line of research may also validate the 8-

ARM as a tool not only for memory assessment, but for gauging decision-making efficiency and flexibility in large animal models.

Conclusions

Across the preceding chapters, evidence was provided that Ossabaw pigs fed a Western Diet for 16 weeks developed measurable cognitive deficits alongside alterations in blood biomarkers. These findings contribute to a growing understanding of how early-stage obesity impacts swine physiology and behavior, offering translational relevance to both human biomedical models and commercial swine production. Given the negative effects of obesity observed in this study, particularly regarding cognitive function and systemic health, there is a clear need to explore dietary strategies that can prevent or mitigate these outcomes. The use of sorghum kombucha is still underway, but following the results of our labs' analysis, further investigation into its application to the swine industry should be conducted. It might be beneficial to investigate best practices for administration, dosing protocols, and long-term effects. Considering the ongoing challenges of food insecurity and the obesity paradox, future research should continue to explore sorghum-based solutions, building on the foundation established by the findings of this thesis, paired with our lab's results.

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Tables

Table 4.1 Sequencing of the 8-Arm Radial Maze outlining various navigation strategies used by rodents, ranging from random arm selection to sequenced movement patterns and advanced spatial mapping. These strategies reflect differing levels of cognitive processing during spatial memory tasks.

Sequencing Strategy	Description	Authors
Random Choice	The most basic and least efficient level of spatial performance. The subject makes choices with no distinct pattern or strategy and acts without a sense of the overall spatial environment.	(Staddon, 1983)
Sequence “1”	The subject moves either clockwise or counterclockwise in one direction, entering each consecutive arm after the other to search for the reward(s).	(Roberts and Dale, 1981; Innis and Macgillivray, 1987; Foreman and Gillett, 2013).
Sequence “2”	90-degree turns between arms, always skipping the consecutive arm	(Watts et al., 1981; Foreman and Gillett, 2013).
Sequence “3”	135-degree turns	(Leis et al., 1984; Foreman and Gillett, 2013).
Sequence “4-3”	180-degree movements. The subject(s) enters the arm that is opposite in position from the preceding arm.	(Foreman, 1985; Okaichi and Oshima, 1990; Foreman and Gillett, 2013).
True Spatial Mapping	The subject(s) are no longer simply following a pattern but are actively processing and storing information about their surroundings. Involves complex cognition, as subject(s) understand the relationships between spatial elements, making their movements or decisions based on an internal map	(Staddon, 1983)
Chunking	Subject(s) entering a specific number of arms that contain a more valuable food source than arms that contain a less valuable food source, but this can only be assessed if specific task demands allow.	(Foreman and Gillett, 2013).