

Sorghum kombucha's impact on lean pigs and obese pigs during early weight loss

by

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Abstract

The use of pigs in research has expanded to serving as valuable translational models for humans. This thesis investigates the body composition changes and behavioral effects of fermented sorghum kombucha in lean Ossabaw pigs and obese Ossabaw pigs during early weight loss. Ossabaw pigs are an established model for studying obesity due to their genetic predisposition to store fat under high-calorie diets. For the first 16 weeks after enrollment, pigs were first grown into a lean body type using a standard diet or an obese body type using a hypocaloric, high-fat, high-fructose corn syrup diet (i.e., Western Diet). Then, liquid supplementation was applied while obese pigs underwent a 6-week weight management program (lean pigs had no dietary changes). The liquid supplementation was twice daily administration (1 L per day) of either an isocaloric control solution or fermented sorghum kombucha.

In Study 1, besides the body weight, weekly ultrasound imaging was used to assess body composition, including back fat thickness and loin eye area. Results revealed that fermented sorghum kombucha was associated with reduced average daily gain and increased back fat loss in obese pigs compared to those receiving the isocaloric control. These outcomes suggest that sorghum kombucha may influence adipose tissue metabolism during the early stages of weight loss.

Study 2 explored how the supplement affected feeding motivation, social interaction, and cognitive flexibility using an automated cognitive feeder test. Behavioral observations revealed that obese pigs, during the early weight loss period, receiving sorghum kombucha demonstrated changes in latency to drink, frequency of bowl switching, and decreased dominance indices, suggesting that sorghum kombucha may modulate social interactions and feeding behaviors.

Together, these findings show that fermented sorghum kombucha may influence early physiological and behavioral markers of obesity in pigs, offering insights into nutritional strategies for metabolic health.

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Chapter 1 - Review of Literature

Introduction

Sorghum is a high-priority crop in the United States, which is the top global producer (FAOSAT, 2023). The state of Kansas leads as the nation's top producer (USDA-NASS, 2024). Sorghum is known for its resilience, using six times less water than corn, making it a key resource in sustainable agriculture, especially in drought-prone regions (Teferra and Awika, 2019). Despite sorghum's advantages, less than 2% of U.S. grown sorghum is processed into products for human consumption, with the vast majority directed toward animal feed or ethanol production (McGinnis and Webster, 2024). To promote economic sustainability for sorghum producers, new value-added food products are needed (Deribe and Kassa, 2020). One such innovation is sorghum kombucha, a fermented beverage rich in polyphenols and microbial metabolites that may offer health benefits for humans while expanding market potential for the crop (Banwo et al., 2021; Kiptanui et al., 2022; Olivier et al., 2023).

Sorghum kombucha holds particular promise in addressing the food insecurity and obesity paradox (Gaur, 2024). In many Western societies, limited financial resources do not equate to undernutrition. Instead, low-income populations often face risks of obesity due to limited access to affordable, healthy foods, reliance on calorie-dense processed options, and cycles of food deprivation followed by overconsumption (Drewnowski and Specter, 2004; Leung et al., 2014). Combined with chronic stress, reduced physical activity, and limited healthcare access, these factors contribute to long-term metabolic disruptions (Engin, 2017). As sugary beverages are frequently targeted in dietary interventions (Apovian, 2004; Fowler, 2016), sorghum kombucha emerges as a promising alternative to diet sodas, offering nutritional and

anti-inflammatory compounds such as polyphenols, prebiotics, probiotics, and postbiotics (Singh et al., 2024; Khablenko et al., 2025).

Obesity is not a simple imbalance between calorie intake and expenditure (Bennett et al. 2018). Rather, it is a multifactorial condition influenced by biological, environmental, social, and behavioral factors (Anekwe et al. 2020; Benusic and Cheskin 2021). Pathophysiological changes often begin before a clinical diagnosis is made, marked by subtle shifts in inflammatory signaling, energy regulation, and behavioral responses (Boitard et al. 2014; Panasevich et al. 2018; De A. Boleti et al. 2023). In early weight gain, adipose tissue expansion may remain metabolically stable. However, sustained overnutrition can lead to adipocyte hypertrophy and pro-inflammatory cytokine release, causing systemic inflammation and neuroinflammatory changes that affect behavior and cognition (Gregor and Hotamisligil, 2011; Thaler et al., 2012; Yau et al., 2014). The gut-brain axis also plays a role in this process. Alterations and differences in gut microbiota composition between lean and obese individuals can modulate gastrointestinal hormone secretion and the central reward receptors, which may drive impulsive and binge eating, influence decision-making, and reduce satiety (Van De Wouw et al., 2017; Strandwitz, 2018). Moreover, mealtime behaviors, especially in social settings, are impacted, leading to increased competition for high-value foods and reduced dietary regulation. As such, behavioral changes may be among the earliest indicators of obesity (Schag et al., 2013; Freitas et al., 2018; Meng and Kautz, 2022).

The current study applies a behavioral lens to investigate how both pre-pathological obesity and the early weight loss stage affect feeding motivation, social interaction, and cognition in a pig model. In social environments, pigs must navigate resource competition through agonistic behaviors, which include threats, displacements, and physical contests

(Petherick and Blackshaw, 1987). Contest interactions reveal important information about dominance, motivation, and the cognitive effort involved in securing food (McGlone, 1986; Maffezzini et al., 2023; Oldham et al., 2025). Operant conditioning tasks and contrafreeloading, where pigs must choose between freely available food and food that requires effort, provide a framework to measure curiosity, reward-seeking, and cognitive flexibility (Baldwin and Meese, 1979; Lawrence et al., 1988; De Jonge et al., 2008). In some cases, binge eating behaviors may also emerge, reflecting dysregulation in reward systems (Avena and Bocarsly, 2012; Dingemans et al., 2017; Romei et al., 2020). The Ossabaw pig provides a unique model for this study. With a genetic predisposition for fat storage under calorie excess, the Ossabaw breed closely mimics human metabolic syndrome (Lee et al., 2009; Toedebusch et al., 2014; Matthan et al., 2018). Their manageable size also makes them suitable for behavioral research in social environments (Nelson et al., 2023; Scott, 2025). Additionally, the use of ultrasound allows for non-invasive, longitudinal tracking of body composition, including back fat and lean tissue, instead of relying on body weight alone (Wagner, 2013; Bazzocchi et al., 2016).

The overall goal of this study is to evaluate whether a sorghum-based kombucha beverage can modulate the physiological and behavioral changes associated with early weight loss in obese pig models. By targeting the pre-pathological phase of obesity, this research aims to identify opportunities for intervention during weight loss before chronic metabolic disease becomes established.

Food Insecurity and The Obesity Paradox

Particularly among low-income and underprivileged groups, food poverty is a major and ongoing public health issue influencing millions of people globally (Swinburn et al., 2019). Food insecurity in the United States is defined by the U.S. Department of Agriculture (USDA) as the

limited or uncertain access to nutritionally appropriate and safe foods resulting from a lack of financial or other resources (Rabbitt et al., 2024). Obesity is complex and goes beyond the “calories in, calories out” philosophy to include anxiety over food availability, poor dietary quality, and disturbed eating patterns (Butler et al., 2024). Based on the USDA statistics, about 17 million American households (i.e., 12.8% of all households) were food insecure in 2022, and in 2023, the percentage was statistically significantly higher (13.5%; Rabbitt et al., 2023; Rabbitt et al., 2024). Food insecurity loads are not shared equally. Households with children (17.9%), single moms (34.7%), Black (23.3%) and Hispanic (21.9%) populations, and those living in urban poverty (15.9) or rural isolation (15.4%) particularly suffer disproportionately (Coleman-Jensen et al., 2022; Rabbitt et al., 2023; Rabbitt et al., 2024). Though usually expressed in financial terms, food insecurity reflects more general systematic inequalities like inadequate access to education, steady job, transportation, and healthcare services, all of which affect nutritional decision-making and long-term health effects (Ver Ploeg et al. 2009; Perrin et al. 2020; Odoms-Young et al. 2024).

In wealthy nations, food insecurity is linked to a 21% increase in obesity risk for the entire population and a 50% increase risk for women and girls (FRAC, 2010; Morales and Berkowitz, 2016; Rezaei et al., 2024). The food insecurity-obesity paradox subverts conventional wisdom in public health that food insecurity always results in undernutrition and low body weight (Dinour et al., 2007). Rather, several studies have found that those with limited access to food are more likely to develop metabolic dysregulation, extreme weight gain, and related chronic illnesses such as type 2 diabetes and hypertension (Franklin et al. 2012; Carvajal-Aldaz et al., 2022). Economic and environmental restrictions that force food-insecure people toward calorie-dense, low-cost meals help to explain the contradictory link. Though diets are generally

deficient in vital nutrients like fiber, protein, and micronutrients, these products, processed snacks, refined grains, and sugar-sweetened beverages, are readily available and offer low-cost satiety (Louzada et al. 2015; Rauber et al. 2015). These eating habits cause too many calories to be consumed without satisfying fundamental nutritional requirements, resulting in the growth of fat mass and a sacrifice of general health (Monteiro et al. 2018; Hall et al. 2019).

Those with sporadic food availability may use coping mechanisms like meal skipping, overindulging during times of food availability, and, if at all possible, bingeing on low-nutrient, high-calorie items (Dinour et al., 2007). Erratic eating habits throw off metabolic homeostasis, aggravate insulin resistance, and stimulate fat storage (Franklin et al., 2012). Long-term alterations in appetite-regulating hormones linked to these feast-famine cycles have been shown in studies to drive increasing hunger, decreased satiety, and stronger cravings for high-energy foods (Dhurandhar, 2016). Furthermore, aggravating weight gain is persistent stress brought on by food insecurity (Sinha and Jastreboff, 2013). The stress reaction raises cortisol concentrations and activates the hypothalamic-pituitary-adrenal (HPA) axis, therefore promoting visceral fat formation and raising the risk of stress-induced eating (Adam and Epel, 2007). Often co-occurring in food-insecure households, psychological stress, including anxiety and depression, helps to explain emotional eating and low motivation for physical activity, hence strengthening obesity in a feedback loop (Leung et al., 2014).

In the food insecurity-obesity link, structural and environmental elements play a significant role (Drewnowski and Specter, 2004). Many food-insecure households reside in "food deserts," geographic locations with limited availability to reasonably priced, nutritious items such as whole grains, veggies, and fresh fruits (Ver Ploeg et al., 2009; Walker et al., 2010). Convenience stores and fast-food restaurants predominate in these neighborhoods, providing

mostly processed, high-calorie choices (Powell et al., 2007). Restricted mobility, time limits, and inadequate kitchen facilities all further make cooking healthy meals less feasible (Zick et al., 2011). For single-parent homes and those juggling several jobs, when financial restrictions and time poverty interact, these obstacles especially apply. In circumstances when better food options are theoretically accessible, they could nevertheless be out of reach depending on cost or cultural mismatch (Darmon and Drewnowski, 2008). These structural obstacles restrict freedom in food choices and help to create a cycle of ill health, stress, and dietary inadequacy (Alaimo et al., 2001; Gundersen and Ziliak, 2015).

To lower the frequency and effects of food insecurity, federal aid programs as the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) and the Supplemental Nutrition Assistance Program (SNAP) were instituted. In 2023, SNAP served approximately 42 million Americans, giving qualified people monthly payments for food purchases (USDA Food and Nutrition Service, 2024). Through specific healthy food packages and nutrition education, WIC helps low-income pregnant women, new moms, and young children. Although these initiatives clearly have enhanced food availability, their effect on diet quality and obesity prevention is dubious (Andreyeva et al., 2015). Purchased processed meals and sugar-sweetened beverages using SNAP assistance could unintentionally encourage poor eating habits. On the other hand, WIC has instituted more focused dietary recommendations that have resulted in higher fruit and vegetable intake and better buying patterns (Whaley et al. 2012; Kreider et al., 2016). While structural interventions like SNAP and WIC aim to mitigate the immediate impacts of food insecurity and obesity, they do not fully address the biological mechanisms of obesity development. To better inform prevention and treatment strategies, it is crucial to understand

how obesity progresses metabolically, often beginning subtly before clinical symptoms emerge (Hotamisligil, 2006).

Different Stages of Obesity

Obesity develops gradually, long before clinical symptoms become apparent. The development of obesity can be categorized into pre-clinical, clinical, and pathological stages (Table 1.1; Egerod et al., 2025). The stages are defined by a continuum of metabolic disturbances, inflammatory signaling, and altered body composition. Without timely intervention, the condition may evolve into irreversible disease states (Hotamisligil, 2006). Early obesity, considered a pre-pathological or preclinical stage, marks the initial accumulation of adipose tissue due to excess energy intake (Table 1.1). During early obesity, metabolic flexibility is still largely intact, and clinical signs are often absent or limited to mild weight gain. However, early metabolic alterations begin to emerge, including mild insulin resistance, adipocyte hypertrophy, and subclinical inflammation (Hotamisligil, 2006; Blüher, 2009). At this early point, enlarged adipocytes begin to secrete small amounts of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6). Although minimal, these secretions mark the start of a shift toward metabolic dysfunction (Hotamisligil, 2006). Additionally, resident M2 macrophages, which normally support homeostasis, may begin to recruit inflammatory M1 macrophages in response to stress signals (Weisberg et al., 2003). The metabolic alterations can occur even in individuals with a normal body index (BMI; 18.5-24.9%), a reason that makes early obesity overlooked using standard anthropometric tools. For instance, individuals classified as “normal-weight obese” (18.5 – 24.9 kg/m²), those with normal BMI but elevated visceral fat and pro-inflammatory markers, may remain undiagnosed (De Lorenzo et al., 2006; Romero-Corral et al., 2010; Nuttall, 2015). Although they provide more

insight, traditional metrics like waist circumference and waist-to-hip ratio also vary depending on population-specific cutoffs and observer consistency (Lean et al., 1995; World Health Organization, 2011).

Metabolic dysregulation, or the subclinical stage, occurs as adipose tissue expands beyond its storage capacity, leading to lipid spillover into ectopic sites such as the liver, contributing to hepatic steatosis and worsening insulin resistance (Table 1.1). At this point, cytokine secretion is amplified, particularly TNF- α , IL-6, and monocyte chemoattractant protein-1 (MCP-1), which recruits more monocytes into adipose tissue (Gregor and Hotamisligil, 2011). The monocytes differentiate into M1 macrophages, further amplifying local inflammation. Inflammation becomes a feedback loop, accelerating adipocyte dysfunction and insulin resistance (Thaler et al., 2012; Yau et al., 2014). Clinical signs during metabolic dysregulation may include fatigue, increased waist circumference, and elevated fasting insulin or glucose levels, even if still within normal diagnostic ranges (Eckel et al., 2005). Consequently, metabolic syndrome represents the clinical stage, characterized by diagnostic metabolic abnormalities such as hyperlipidemia, elevated blood pressure, and impaired glucose tolerance (Table 1.1). At this stage, most tissues within the body have some degree of insulin resistance, and inflammation becomes chronic, as macrophage infiltration intensifies, and cytokine concentrations within the tissues remain elevated. Additionally, immune cells like T cells and B cells may contribute to the chronic inflammatory state (Winer et al., 2011). Excess abdominal fat becomes more prominent, and individuals may receive formal diagnoses of metabolic syndrome or prediabetes (Després, 2006; Neeland et al., 2019).

Chronic obesity reflects long-term metabolic stress resulting in tissue damage and irreversible dysfunction. The adipose tissue immune environment is dominated by pro-

inflammatory M1 macrophages, and cytokines like TNF- α , IL-6, and C-reactive protein (CRP) circulate systemically at high levels (Gregor and Hotamisligil, 2011; Thaler et al., 2012; Yau et al., 2014). Persistent inflammation, oxidative stress, and lipotoxicity contribute to the progression of type 2 diabetes mellitus (T2DM), non-alcoholic steatohepatitis (NASH), and cardiovascular disease (CVD; Samuel and Shulman, 2016; Opio et al., 2020). Clinical management during this stage often involves pharmacological and surgical interventions, although organ damage and impaired quality of life are common (Lowell and Shulman, 2005; Patti and Corvera, 2010). Finally, morbid obesity, or advanced pathological obesity, is defined by multi-organ complications, systemic inflammation, and high disease burden (Table 1.1). The advanced pathological stage is often accompanied by sarcopenic obesity, mobility limitations, and end-organ failure, leading to reduced quality of life and increased mortality risk (Montani et al., 2015).

Notably, even modest weight loss of 5-10% of total body weight has been shown to reverse many of the physiological impairments associated with obesity, including improved insulin sensitivity and lower blood pressure (Wing et al., 2011; Knowler et al., 2002). For example, a person who weighs 200 pounds would only need to lose 10 to 20 pounds to begin experiencing measurable health improvements. However, during the chronic and morbid stages of obesity, sustained inflammation, immune cell infiltration, and ectopic fat accumulation often lead to permanent alterations in organ function, including hepatic fibrosis and cardiovascular remodeling (Wellen and Hotamisligil, 2005; Francque et al., 2016; Saltiel and Olefsky, 2017). While at these advanced stages, lifestyle interventions can still offer benefits, such as slowing progression and reducing symptom severity, complete reversal becomes less likely, and pharmacological or surgical therapies are often required for meaningful outcomes (Mingrone et

al., 2012; Apovian, 2016). The opportunity to reverse metabolic disturbances is greatest during the early obesity and metabolic dysregulation stages, when adipose tissue remains plastic and inflammatory responses may still be modifiable (Blüher, 2009; Dattilo et al., 2012; Haire-Joshu and Tabak, 2016).

While much attention has focused on the physical and metabolic consequences of obesity, its impact on the brain is increasingly recognized as an area of concern. Disruptions in inflammatory and hormonal signaling associated with obesity extend beyond peripheral tissues, influencing cognitive function and behavior (Prickett et al., 2015). The neurobiological alterations have been documented across species and may contribute to the persistence and complexity of obesity (Knecht et al., 2008; Sharma and Fulton, 2013).

Obesity and Cognitive Function: Neurobiological and Behavioral Impacts

Research within the last decade highlights a bidirectional relationship between obesity and cognitive dysfunction, in which excess adiposity not only results from maladaptive behavioral regulation but also contributes to long-term alterations in brain structure and function (Kanoski and Davidson, 2011; Francis and Stevenson, 2013; Cheke et al., 2016). These neurocognitive consequences extend beyond simple associations with metabolic syndrome, suggesting that obesity itself plays a mechanistic role in impairing learning, memory, attention, and executive function (Smith et al., 2011; Fitzpatrick et al., 2013). At the neurobiological level, obesity is associated with low-grade systemic inflammation, insulin resistance, and oxidative stress, which can cross the blood-brain barrier and affect neuronal integrity (Rohm et al., 2022; Naomi et al., 2023). Inflammatory cytokines such as TNF- α , IL-6, and C-reactive protein, elevated in obese individuals, have been shown to disrupt synaptic plasticity, reduce neurogenesis, and impair long-term potentiation (LTP), particularly within the hippocampus, a

brain region critical for memory consolidation (Jeon et al., 2012; Thaler et al., 2012; Miller and Spencer, 2014).

Animal models have been instrumental in delineating these mechanisms. In mice fed a high-fat diet, hippocampal neurogenesis was significantly reduced and neuroinflammation increased, which was accompanied by spatial memory deficits and increased markers of neuroinflammation (Murray et al., 2009). Similarly, Yucatan minipigs have shown impairments in learning tasks and altered exploration patterns following obesity induction, consistent with findings in rodent models (Gautier et al., 2020). These impairments appear to be tied to both systemic metabolic dysfunction and altered brain-derived neurotrophic factor (BDNF) signaling, a key regulator of neuronal health and plasticity (Molteni et al., 2002; Gómez-Pinilla, 2008; Lukkahatai et al., 2025). In humans, neuroimaging studies have revealed that individuals with obesity often exhibit reduced gray matter volume in the prefrontal cortex and hippocampus, which correlates with poor performance in executive function tasks (Gustafson et al., 2003; Fernández-Andújar et al., 2021). Obesity in childhood and adolescence is particularly concerning, as it coincides with critical periods of brain development, potentially resulting in long-term cognitive vulnerabilities (Liang et al., 2014).

Moreover, accumulating evidence suggests that these neurocognitive changes may be compounded by disruptions in the gut-brain axis (Cryan and Dinan, 2012; Bruce-Keller et al., 2015). Obesity-induced alterations in gut microbiota composition can influence central nervous system function via microbial metabolites, vagal nerve signaling, and modulation of systemic inflammation (Cryan et al., 2019). The gut-brain communication plays a pivotal role in regulating reward processing, appetite, and mood, systems frequently dysregulated in individuals with obesity. For example, obesity has been linked to enhanced dopamine signaling in the

nucleus accumbens, contributing to heightened reward sensitivity and compulsive food-seeking behaviors (Volkow et al., 2013).

Rather than viewing obesity solely as a metabolic or physical disorder, researchers are increasingly reporting that the condition involves central nervous system impairment (Smith et al., 2011; Morys et al., 2024; Pirozzi et al., 2025; Saeed et al., 2025). Understanding these connections may facilitate the development of integrative, brain-targeted interventions that address both behavioral and metabolic regulation (Satyal et al., 2021). Building upon this neurobiological perspective, researchers also recognize that obesity is fundamentally a behavioral condition, shaped by the interplay of neurological, psychological, and social factors (Marks, 2015; Farr et al., 2016).

In social environments, obese people may encounter prejudice or discrimination that influences their involvement in group activities, physical mobility, or even contacts in healthcare settings (Puhl and Heuer, 2009). These social and cognitive obstacles lower participation in activities that promote health, therefore generating a reinforcing cycle that prolongs negative results (Smith et al. 2011). Furthermore, research implies that people with obesity may have changed the reward value of food, which would result in changing patterns of expectation, response, and satisfaction while eating (Stice et al., 2008). Including behavioral science in obesity research is needed, especially in the design of treatments aiming at not just what people eat but also why and how they eat (Hall and Kahan, 2018).

Obesity and Behavior

Developing successful preventive and treatment plans for obesity depends on an awareness of the behavioral aspects, especially as the food environment, cultural standards, and personal reward sensitivity determine many of the behaviors leading to weight increase

(Montmayeur, 2009). Particularly, the dopaminergic pathways implicated in motivation, pleasure, and impulse control, human eating behavior is intimately related to the reward system of the brain (Berthoud, 2012). This system may be changed in those with obesity, resulting in increased sensitivity to food cues, more intense desires for high-calorie foods, and poor satiety control (Volkow et al., 2013). Particularly in settings surrounded by readily available, energy-dense foods, these neurobiological alterations provide a feedback loop in which hedonic eating is rewarded, often overrunning internal hunger and fullness signals (Adams et al., 2019). Importantly, the fast-food industry's financial power and aggressive marketing strategies play a critical role in shaping food preferences, particularly by targeting vulnerable populations with advertisements for high-calorie, ultra-processed products (Grier and Kumanyika, 2008; Harris et al., 2010).

New studies on pharmacological weight loss treatments have drawn fresh focus on the behavioral foundations of obesity (Müller et al., 2022). Semaglutide and other glucagon-like peptide-1 (GLP-1) receptor agonists have been found to lower hunger, increase satiety, and cause appreciable weight loss in people with type 2 diabetes or obesity (Wilding et al., 2021). Intriguingly, patients on GLP-1 drugs sometimes describe changes in taste preferences, including a lower demand for sugary or highly processed foods and an appreciation for more nuanced and less sweet flavors (Brindisi et al., 2019; Khan and Doty, 2025). Pharmaceutical treatments or successful weight loss might change the brain circuits involved in food reward, therefore offering a chance to reset behavioral patterns causing obesity (Van Bloemendaal et al., 2014). Still, these interventions, like the GLP-1 drugs, need to complement rather than replace sustainable improvements in food choices, exercise, and environment (Bray and Ryan, 2012). The neurobehavioral landscape may also elevate the importance of offering less sweet, yet still

palatable beverage options during weight loss interventions, particularly as individuals begin to shift away from high-sugar preferences (Dilley et al., 2025).

The part sugar-sweetened beverages play in causing poor metabolic health and excessive calorie consumption raises additional issues as well (Malik et al., 2010). Particularly among teenagers and young adults, sugar-sweetened beverages rank as the main source of added sugars in the American diet, according to the Centers for Disease Control and Prevention (CDC, 2022). Sugary drinks typically remain a comfort and familiar source for those switching to better diets or using appetite-modulating drugs; they are therefore tough to cut out (Gearhardt et al., 2011). Some studies imply that, especially in early phases of dietary change, participation in programs like the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) may momentarily boost the intake of sweetened beverages by encouraging the substitution of sodas with sugary fruit-based products (Tester et al., 2016). While WIC aims to promote healthier dietary choices, it frequently permits or even encourages the substitution of soda with 100% fruit juice, often considered a healthier alternative, even though fruit juice can still contribute high amounts of sugar and calories. As a result, early in dietary transitions, overall sugar intake from beverages may not decrease significantly, despite reduced soda consumption (Grap et al., 2024). National data from the CDC's National Survey of Children's Health (2021) further reveal that over half of children aged 1–5 years consume sugar-sweetened beverages at least once weekly (Hamner et al., 2023). Especially during programs or interventions targeting beverage intake among vulnerable groups, it is important to offer culturally appropriate, nutritionally balanced alternatives (Bleich et al., 2018).

Kombucha: A Culturally Relevant Beverage for Metabolic Health

Kombucha, a fermented tea with probiotic potential, has emerged as a promising functional beverage that may serve in weight management strategies or maintaining a healthy bodyweight (Fraiz et al., 2024). Traditionally produced from sweetened tea, kombucha is a fermented beverage created by a symbiotic culture of bacteria and yeast (SCOBY). Kombucha's supposed health advantages, especially in digestive health, immunological support, and inflammation reduction, have helped it become more well-known over recent decades (Su et al., 2024; Fraiz et al., 2025). During the fermentation process, carbohydrates are converted into organic acids, ethanol, and several bioactive metabolites, including acetic, gluconic, lactic, and glucuronic acids (Jayabalan et al., 2014). While the microbial profiles of kombucha might vary greatly depending on substrate, fermentation period, and environmental circumstances, the presence of metabolites is supposed to impart probiotic-like advantages (Jayabalan et al., 2014; Selvaraj and Gurumurthy, 2024).

Acetic acid possesses antimicrobial properties that help inhibit the growth of pathogenic bacteria and yeasts, both during fermentation and potentially within the gastrointestinal tract by contributing to intestinal barrier integrity by lowering intestinal pH, which can inhibit harmful gut bacteria and support beneficial microbes (Greenwalt et al., 1998; Marco et al., 2017). Gluconic acid may aid in mineral absorption and enhance gut health through its mild acidifying action (Battikh et al., 2012). Lactic acid supports gastrointestinal balance and is commonly associated with probiotic function in fermented foods (Marsh et al., 2014). Glucuronic acid plays a detoxification role in the liver, potentially supporting the body's natural ability to eliminate xenobiotics and metabolic waste (Viciomini et al., 2024). In addition to organic acids, kombucha retains the tea's natural polyphenols, which are known for their antioxidant, anti-

inflammatory, and metabolic-regulating properties. Fermentation can enhance the bioavailability of these compounds, further contributing to kombucha's proposed health benefits (Villarreal-Soto et al., 2018; Sinir et al., 2019).

Another crucial distinction in kombucha production is the role that aerobic versus anaerobic fermentation plays (Villarreal-Soto et al., 2018). Kombucha is usually brewed under aerobic conditions, where oxygen availability supports the formation of a cellulose-based biofilm at the air–liquid interface, a defining feature of a symbiotic culture of bacteria and yeast (SCOBY; Marsh et al., 2014). The SCOBY is composed primarily of acetic acid bacteria such as *Komagataeibacter xylinus* and yeasts like *Zygosaccharomyces* and *Saccharomyces*, which work in concert to ferment sugars and synthesize bioactive compounds (Srither et al., 2025). Acetic acid bacteria include *Gluconacetobacter xylinus*, which generate acetic and gluconic acid, and find a home in the oxygen-rich environment (Jayabalan et al., 2014). Many other fermenting techniques, including those used to make lactic acid beverages, happen under anaerobic circumstances and produce distinct microbial and metabolic profiles (Tamang and Kailasapathy, 2010).

Beyond the biological activity, kombucha combines traditional fermenting methods with growing interest in sustainable, plant-based, and culturally flexible diet practices as functional beverage research develops (Popkin et al., 2006; Villarreal-Soto et al., 2018). As a fermented beverage, kombucha can be an affordable, gluten-free, and culturally adaptable alternative for populations vulnerable to food insecurity or metabolic disease (Sreeramulu et al., 2000; Taylor and Emmambux, 2008; Hur et al., 2014).

Sorghum

Although kombucha has long been produced from green or black tea, increased interest in alternative substrates has driven researchers to investigate other polyphenolic-rich sources like herbal teas, fruit extracts, and cereal grains (Villarreal-Soto et al., 2018). Therefore, sorghum was selected as a candidate with great promise because of its high polyphenolic concentration and functional food possibilities (Sreeramulu et al., 2000; Nelson et al., 2023). Kombucha made with sorghum offers an added advantage: its spent grain byproduct remains nutritionally dense and may be repurposed into whole grain snacks or livestock feed, thus linking fermentation to sustainable grain use (Mussatto & Roberto, 2006; FAO, 2018).

Though most of sorghum is used for animal feed and biofuels, sorghum is already grown extensively in the United States from an agricultural and commercial standpoint (Awika and Rooney, 2004). Sorghum is particularly adapted for arid and semi-arid regions, including sub-Saharan Africa, India, and the southern United States, due to its resistance to drought, heat, and poor soil conditions, among the top five cereal crops worldwide in terms of acreage and yield, sorghum is absolutely important for sustainable agriculture and food security (Hao et al., 2021; Yahaya and Shimelis, 2022). With most of the sorghum grown in Kansas, Texas, and Nebraska, United States growers produced 430 million bushels of sorghum in 2021 (FAO, 2021). The USDA claims that a great untapped resource for functional food innovation is there, as over 90% of the sorghum grown locally does not find use in human food chains (U.S. Grains Council, 2022; USDA, 2023). Growing demand for human-grade sorghum products, including kombucha and its variants, may help improve the economic viability of sorghum farming and better match agricultural policy with public health objectives (Awika and Rooney, 2004; Taylor and Emmambux, 2008). Particularly in the Midwest and Southern areas where sorghum is a major

crop, small-scale kombucha production utilizing locally farmed sorghum could be very advantageous for rural economies (USDA Rural Development Reports, 2024).

Rich nutritional and phytochemical profile of sorghum is one of its defining qualities. Whole grain sorghum keeps its bran layer, which has a range of beneficial components like polyphenols, flavonoids, and phenolic acids, unlike refined grains. Sorghum is a candidate for inclusion in functional meals targeted at enhancing metabolic health since these chemicals are linked with antioxidant, anti-inflammatory, and perhaps anti-obesity properties (Awika and Rooney, 2004). Furthermore, high in dietary fiber, sorghum helps to regulate glucose levels and promotes intestinal health (Links et al., 2015). Sorghum also has a quite low glycemic index when compared to other cereal grains, which qualifies for those at risk for type 2 diabetes or insulin resistance (Sila et al., 2012). From a nutritional standpoint, sorghum has approximately 70 percent of carbs, 11 percent of protein, and 3.5% percent of fat, though these figures vary somewhat depending on cultivar and processing technique (Kulp and Ponte, 2000).

Those with celiac disease or gluten intolerance will find Sorghum particularly appealing because of its structure and composition (Ciacci et al., 2015). Unlike wheat, barley, and rye, which lack gluten-forming proteins, sorghum is naturally gluten-free and appropriate for a growing portion of consumers following gluten-free diets (Schober et al., 2005). Food companies investigated alternative grains that not only satisfy dietary requirements but also provide nutritional value in response to consumer demand for gluten-free products (McKevith, 2004). Though widely available in mainstream supermarkets, niche stores, and internet markets now increasingly feature sorghum flour, sorghum flakes, and popped sorghum. Moreover, sorghum can be produced into several reasonably priced, culturally relevant food products like beverages, flatbreads, extruded snacks, and porridges (Xiong et al., 2019; De Kock et al., 2024). These uses

not only vary the commercial potential of the grain but also fit more general objectives of enhancing dietary diversity and nutrition security in both low- and high-income environments (Taylor and Emmambux, 2008; FAO, 2017).

Regarding phenotypic variation, sorghum comes in a number of colors, structures, and polyphenolic compositions. Commonly grown are red, brown, black, and white sorghums, colored types usually show more polyphenols. Red (i.e., Sumac), brown and black sorghum grains, for example, sometimes include condensed tannins, anthocyanins, and 3-deoxyanthocyanidins, which help to explain their antioxidant capacity and possible anti-diabetic effects (Dykes and Rooney, 2006). The particular cultivar chosen affects not just nutritional composition but also sensory qualities, including flavor, texture, and astringency, which might impact consumer acceptance and formulation techniques (Choi et al., 2019). Red sumac sorghum, with its unique reddish hue and high polyphenol concentration, is one kind of especially interesting subject for this current work (Kosar et al., 2007). Although white sorghum is usually preferred for culinary uses because of its neutral taste, red variants have more health advantages and show great prospects for functional food development (Taylor and Duodu, 2017). While darker varieties such as black sorghum may offer greater antioxidant potential, the current study, for an initial product development, prioritized flavor acceptability to ensure subjects' palatability, though black sorghum remains a promising candidate for future formulation refinement.

Fermented Sorghum Kombucha: Functional Potential Beverage

Despite sorghum's potential as a functional beverage, the use of sorghum as a kombucha substrate remains largely unexplored, with only a few studies evaluating its nutritional, metabolic, and sensory benefits (Lopes et al., 2019; Kiptanui et al., 2022). The sorghum's

kombucha research gap provides an opportunity to investigate how fermentation may enhance sorghum's bioavailability, antioxidant potential, and applications beyond traditional uses. During fermentation, sorghum's natural sugars and polyphenols are metabolized by a symbiotic culture of bacteria and yeast (SCOBY), like in traditional kombucha, producing key organic acids, such as acetic, gluconic, and lactic acids, while breaking down complex polyphenolic compounds into smaller, more bioavailable metabolites that may increase antioxidant activity and bioaccessibility (Hur et al., 2014; Yeo and Ewe, 2015). Although the specific microbial interactions with sorghum's tannins and phenolic acids remain underexplored, these compounds provide a rich substrate for fermentation (Kiptanui et al., 2022).

During fermentation, antinutritional compounds such as tannins, which can limit amino acid availability, are reduced by up to 63.1%. The tannin breakdown process enhances the nutritional profile of sorghum, making it a more effective substrate for improving protein digestibility (Hassan and Tinay, 1995; Lopes et al., 2019). In cultures where grain-based diets predominate and protein quality is a nutritional issue, this can especially be helpful (Millward, 1999). Additionally, kombucha's natural effervescence and acidity may satisfy sensory cravings for carbonated drinks while delivering significantly less sugar. After fermentation, kombucha typically contains less than 6 g of sugar per 100 mL, compared to over 10 g in soda, which has been linked to metabolic dysregulation and excessive caloric intake (Villarreal-Soto et al., 2018; CDC, 2022). Substituting kombucha for just one liter of soda per day can reduce sugar intake by over 40 g, potentially lowering daily glycemic load and overall energy consumption (Malik et al., 2010).

The bioactive compounds produced through kombucha fermentation, particularly phenolic acids, play a critical role in combating metabolic syndrome and obesity-related

conditions by acting as antioxidants and inflammation modulators (Awika and Rooney, 2004). Sorghum-based kombucha may be especially potent in this regard due to its higher concentration of polyphenols. These compounds have the potential to reduce oxidative stress and downregulate pro-inflammatory cytokines in adipose tissue, potentially interrupting the progression from chronic low-grade inflammation to full metabolic dysfunction (Lee et al., 2022; Tran et al., 2024). Significantly, the fermentation process enhances the bioavailability of these polyphenols, transforming them into more physiologically active forms. As a result, sorghum kombucha may deliver synergistic benefits that surpass those of non-fermented sorghum or traditional tea-based kombucha (Moraes et al., 2012; De Sousa et al., 2019; Francis et al., 2019). In addition to its health-promoting properties, sorghum kombucha also offers a sustainability advantage. Unlike tea-based kombucha, which produces substantial post-steeping waste, sorghum kombucha is made from whole grains. The residual sorghum can be repurposed into snacks, porridges, or baked goods, reducing food waste and maximizing the nutritional yield per unit of input (Mussatto and Roberto, 2006; FAO, 2017).

Ossabaw Pig for Studying Obesity

Given the nutritional and functional potential of fermented sorghum kombucha demonstrated *in vitro*, and through compositional analyses (Hassan and Tinay, 1995; Lopes et al., 2019; Kiptanui et al., 2022), sorghum kombucha's impact on the physiology and behavior using a relevant biomedical model is essential. The Ossabaw pig serves as one of the most medically relevant models for investigating obesity and metabolic syndromes due to their particular genetic and physiological traits (Dyson et al., 2006). Originating from wild pigs kept apart on Ossabaw Island, Georgia, for hundreds of years, these creatures developed in an environment marked by food shortage and sporadic famine (Leigh et al., 2004; Weiss, 2011;

Zhang et al., 2021). They so evolved what is known as the "thrifty genotype," an observation first described by Neel (1962), which describes a genetic inclination to effectively store energy in the form of fat during times of caloric abundance to endure future shortage. The thriftiness feature makes Ossabaw pigs quite prone to weight increase, especially visceral fat deposition, in modern research environments when given high-fat, high-energy diets. Their sensitivity to dietary composition, especially under obesogenic conditions, allows scientists to replicate in a controlled experimental model human-like paths of obesity, prediabetes, and insulin resistance (Dyson et al., 2006; Lee et al., 2009; Neeb et al., 2010; Suzuki et al., 2011; Sturek et al., 2020).

Ossabaw pigs are especially beneficial for research trying to grasp the early phases of metabolic dysregulation because they show obvious changes from preclinical to pathological conditions (Dyson et al., 2006). These pigs routinely exhibit classic traits of human metabolic syndrome, including increased adiposity, hypertension, dyslipidemia, and hyperinsulinemia, when fed a Western-style diet heavy in fats and carbohydrates (Lee et al., 2009; Neeb et al., 2010). Ossabaw pigs develop obesity naturally from environmental exposure, unlike rodent models, which generally show only partial features of these diseases or require genetic alteration to express metabolic problems (Spurlock and Gabler, 2008). Studies on nutrition and lifestyle interventions, such as food modification or microbiome-targeted treatments, have greater implications for clinical research than rodent models when the Ossabaw pig is used (Elmadhun et al., 2012; Mouzaki et al., 2013). Moreover, Ossabaw pigs are a perfect link between fundamental animal research and therapeutic translation since porcine and human cardiovascular, digestive, and immunological systems are similar (Spurlock and Gabler, 2008).

Using the Ossabaw model also has advantages for longitudinal research tracking the evolution of obesity and associated comorbidities over time (Dyson et al., 2006). Researchers

can put Ossabaw pigs under experimental circumstances for long periods thanks to their reasonable size and modest development rate, therefore enabling thorough monitoring of physiological, metabolic, and behavioral changes (Spurlock and Gabler, 2008). This is especially helpful for assessing how early-life dietary exposures affect long-term health outcomes, a major emphasis area for both pediatric obesity research and preventative nutrition science.

Furthermore, their sensitivity to nonalcoholic fatty liver disease (NAFLD), a developing issue in obese human populations, offers chances for researching liver-specific diseases not effectively reproduced in rats (Lee et al., 2009).

Apart from its relevance as a metabolic model, the Ossabaw pig offers the opportunity to consider the behavioral aspects of obesity and nutritional control. The Ossabaw's natural, food-conserving evolutionary background determines not only its physiological reactions to nutrition but also its behavioral features (Spurlock and Gabler, 2008; Castanon et al., 2015). Likely influenced by generations of adaptation to feast-or-famine conditions, epigenetic programming may help explain variations in how Ossabaw pigs forage, search, and eat food relative to commercial breeds (Waterland and Michels, 2007). These pigs may show more persistent foraging activities, higher reward sensitivity, and more feeding motivation, qualities that match maladaptive eating patterns seen in human groups suffering dietary instability (Neel, 1962; Di Renzo et al., 2016). In this regard, their behavioral profile becomes a crucial component of knowledge of obesity beyond biochemical mechanisms, combining the neurological and contextual influences on food-related decision-making (Castanon et al., 2015). Particularly because of their limited domestication and selection for fast development, behavioral responses in Ossabaw pigs may also differ greatly from those of typical commercial pigs (Stolba and Wood-Gush, 1989; Gustafsson et al., 1999; Held et al., 2005).

Ultrasound Assessing Obesity and Body Composition

Following the use of the Ossabaw pig as a biomedical model, objective measurement of body composition is essential for evaluating interventions such as fermented sorghum kombucha. Ultrasound imaging offers a reliable, non-invasive alternative to conventional measures like body mass index (BMI) and waist circumference or more invasive measures such as imaging (e.g. PET scans), when animals need to undergo anesthesia. Ultrasound provides direct insight into parameters such as back fat thickness and loin eye area (lean mass; Wagner, 2013). Although clinical measures (i.e., BMI) offer helpful screening instruments, they sometimes overlook important physiological differences, including the amount and distribution of lean mass and fat mass (Wells, 2005). On the other hand, ultrasonic waves allow for real-time imaging and quantification of subcutaneous and, in some cases, intramuscular fat layers (Scholz et al., 2015). Ultrasound appeals because of its safety, portability, and somewhat low cost relative to computed tomography or magnetic resonance imaging, which, although more exact, are prohibitively costly and depend on specific infrastructure (Ponti et al., 2020). A major benefit of ultrasound in long-term health monitoring or experimental studies is its ability to be used repetitively and longitudinally (Perkisas et al., 2018).

Ultrasound has been used in clinical human research to gauge subcutaneous fat thickness at several anatomical sites, including the abdomen, thigh, and upper back, enabling regional assessments of fat accumulation that might have more predictive value for metabolic risk than generic measures like BMI (Wagner, 2013). Found surrounding internal organs, visceral fat has been proven to have higher relationships with insulin resistance, systemic inflammation, and cardiovascular illnesses than peripheral fat (Després, 2006; Neeland et al., 2019). Although anatomical depth and variability in abdominal structures make imaging visceral fat directly using

ultrasonic more difficult, techniques for measuring abdominal wall thickness or evaluating intra-abdominal echogenicity have shown interesting relationships with metabolic risk profiles.

Ultrasound-based measurement strategies, alongside common probe types and measurement sites, are especially crucial in spotting those with normal-weight obesity, a normal BMI but excessive visceral fat and poor metabolic health (Prado and Heymsfield, 2014).

Ultrasound has long been used in biomedical and agricultural research because of the non-invasive, patient-side measures (Swindle et al., 2012). In commercial animals, it was used to assess carcass quality. Ultrasonography is employed in swine studies to measure characteristics, including back fat thickness, loin eye area, and marbling (Moeller and Christian, 1998). These measurements provide a dynamic view of how nutrition, genetic background, and environment affect adiposity by allowing the estimation of body composition and the tracking of fat and muscle changes over time in live animals (Sather et al., 1996).

The applicability of ultrasonic waves in translational studies adds even more value in obesity research (Spurlock and Gabler, 2008). Underlining its adaptability and simplicity, the method has been applied for decades in agricultural research to guide breeding programs, feeding efficiency, and meat quality criteria (McLaren et al., 1989). From a preclinical and clinical standpoint, increasing the use of ultrasound in health evaluations could provide a more complex understanding of obesity-related hazards among communities not adequately addressed by BMI-based screening (Wells, 2014). Real-time imaging of fat storage can also give non-invasive markers of energy balance and health status in animal welfare science, therefore guiding housing, feeding, and enrichment plans (Gentry et al., 2008). As the field of obesity research evolves, imaging-based tools like ultrasound will continue to play a pivotal role in improving

diagnostic accuracy and monitoring the effectiveness of interventions targeting fat distribution and mobilization (Heymsfield and Wadden, 2017).

Behavioral and Social Dimensions in Pigs

Although imaging technologies like ultrasound enhance the understanding of the physical manifestations of obesity, they are highly correlated with the subject's behavioral and social factors that contribute to obesity outcomes. Though swine's importance in the genesis and management of the disease is well known, behavioral investigations in swine models have long been underused in obesity research (Zhang et al., 2021). Many times, traditional studies focus on physiological indicators, such as weight gain, insulin sensitivity, and lipid profiles, without fully appreciating how behavior patterns around meals, social contact, and environmental response affect metabolic outcomes (Guevara et al., 2022). Particularly, studies on obese pigs have often neglected behavioral features, including reward sensitivity, impulsive decision-making, and emotional reactivity, all of which have been substantially correlated in humans with obesity (Wangsness et al., 1980). Abundantly evident that as the swine research community starts to extend behavioral assessments, swine models, especially those like the Ossabaw pig, offer great chances to investigate how obesity affects motivation, cognition, and social dynamics (Zhang et al., 2021). Nonetheless, to the author's knowledge, only 4 studies in Ossabaw pigs include behavioral tests or overall activity monitoring (Wangsness et al., 1980; Nelson et al., 2023; Nelson, 2024; Scott, 2025).

The interaction between classical and operant conditioning and food-motivated tasks is one fundamental behavioral feature (Abramson and Kieson, 2016). Pigs can learn, for instance, to link the sound of a pen door or the presence of a researcher with upcoming meal delivery (Gieling et al., 2011). These signals might have reinforcing qualities over time, causing

anticipatory actions, such as standing at the feeder, more vocalizing, or displacement activities (Guevara et al., 2022). In this regard, operant training paradigms, such as paddle-pressing or lever-pulling tasks, offer strong instruments to evaluate drive and decision-making (Patterson-Kane et al., 2008). Measuring latency to respond, persistence of effort, and willingness to switch between food sources can expose variations in how fat and lean pigs rank reward and distribute attention (Wangsness et al., 1978; Wangsness et al., 1980). These behavioral measures may be very helpful in evaluating cognitive inflexibility or impulsivity related to metabolic malfunction since they mimic human investigations employing Skinner boxes or touchscreen-based cognitive tasks (Krause et al., 2021).

Especially when assessing hedonic choices between caloric and non-caloric items, preference testing is a useful approach for investigating diet-related behaviors in pigs (Maselyne et al., 2015). Nelson et al. (2023) showed that despite sour and bitter components, adult Ossabaw pigs not only tolerated but preferred fermented sorghum kombucha over an isocaloric sweetened water solution. Previously, research focused on Hanford miniature pigs presumed that pigs avoid many bitter tastes, showing clear dose-response aversion in preference tests (Nelson and Sanregret, 1997). Pigs may show not only caloric-driven decisions but also possible cognitive interaction with more complex food stimuli, thereby augmenting our knowledge of satiety, motivation, and reward behavior in obesity models (Lind et al., 2007; Gieling et al., 2011).

Although most biomedical research keeps pigs individually and separates feeding for data clarity, real-world situations, especially in agriculture and behavior-oriented welfare studies, involve animals eating in groups, which adds dynamics of rivalry, dominance, and cooperation (Spicer and Aherne, 1987). By observing pigs sharing space around feeders or coordinating feeding schedules, researchers can assess how social dynamics, including dominance and

submission, shape feeding behavior. For instance, Ewbank (1976) studied commercial pigs and observed that dominant individuals consistently gained priority access to feeders, while subordinate pigs exhibited avoidance behavior and reduced intake. Turner and Edwards (2004) used feed competition tests with group-housed finishing pigs and showed that aggressive interactions increased when feeder space was limited, highlighting the role of resource availability in reinforcing social rank. Similarly, Puppe et al. (2008) applied social confrontation tests in juvenile pigs to evaluate the stability of social hierarchies, finding that repeated interactions influenced both aggressive behavior and social cohesion. While subordinate animals might avoid competition, resulting in reduced food consumption, dominant pigs might monopolize access to preferred feeders. Moore et al. (1994), with growing pigs kept in groups, found that socially subordinate pigs had lower growth rates and increased stress behaviors, indicating physiological consequences of social position. Similarly, Samarakone and Gonyou (2008) demonstrated in group-housed barrows that more aggressive pigs maintained greater access to feed, leading to higher body weights and backfat thickness, while subordinate pigs experienced elevated cortisol levels.

Contest behavior is observable during access to preferred locations or feeding times (Maselyne et al., 2015). These contests involve competitive interactions, often classified as agonistic behaviors, which encompass a range of social conflict responses such as threats, displays, chases, and physical confrontations (Lawrence and Terlouw, 1993). Agonistic behavior reflects the animal's position within a social hierarchy and serves as a mechanism for establishing or defending access to resources. The contest term refers to competitive interactions where the outcome determines which individual gains access to a limited resource, such as food or space. In the context of obesity studies, pigs with greater body mass may have an advantage in

physical contests due to their size and strength (Wangsness et al., 1980). The interplay between social rank and physical condition thus provides a valuable lens for interpreting how dominance and submission influence feeding access, metabolic outcomes, and behavioral adaptations. Moreover, tracking changes in agonistic frequency and intensity can help identify early behavioral signs of social withdrawal or emerging obesity-related impairments before overt weight gain occurs (Zhang et al., 2021).

Particularly those that track the number and length of interactions amongst pigs, social index measures offer a way to evaluate social integration and stability within a group (Craig, 1986; Puppe et al., 2008). Crucially, these indices should not be seen as set labels, dominance hierarchies and affiliative connections change with time depending on environmental conditions, stress level, and health state (Bang et al., 2010). By means of a temporal approach to social indexing, researchers can identify dynamic changes in social behavior possibly linked to developmental phases or experimental treatments (Büttner et al., 2019). A pig that starts off as a dominating feeder, for instance, can lose that position if weight increases cause mobility to drop, or if a pen mate becomes more aggressive. These oscillations present a complex picture of how obesity affects not only the individual but also the larger social fabric of the group (Otten et al., 2002; Bayly et al., 2006; Büttner et al., 2020).

Additionally, diagnostic actions are exploratory, especially oronasal ones, such as sniffing, rooting, or nudging. Although these behaviors are sometimes understood as natural foraging ones, under stress or in barren surroundings, they can become extreme or repetitive and turn into stereotypies, but they do not always represent stereotypy (Mason and Mendl, 1997). Aberrant actions, defined by their invariance, lack of clear objective, and repetition, may indicate compromised welfare or frustration. In studies on obesity, more non-nutritive oral behaviors

(NNOB) free from context or variation may point to lower behavioral flexibility or higher anxiety (Wangsness et al., 1980; Arellano et al., 1992; Lawrence and Terlouw, 1993; Bergeron and Gonyou, 1997). Healthy foraging from pathological stereotypies can be distinguished by careful observation and context-based interpretation, ideally informed by reference materials including the Animal Behavior Dictionary (McFarland, 2006). Another action that can help us grasp pig's cognitive, drive, and social involvement is contrafreeloading. Documented in many species, the contrafreeloading phenomenon, where animals choose work for food instead of fighting for available food, is thought to represent an inherent interest in environmental interaction or cognitive stimulation (Osborne, 1977; Inglis et al., 1997).

Contrafreeloading

In a foundational study on contrafreeloading in pigs, De Jonge et al. (2008) tested commercial female piglets (Large White x Landrace) at 31 days of age individually in a two-compartment conditioned place preference setup. Across four experiments, piglets learned to associate one compartment with effortful food acquisition, chocolate raisins hidden in straw, and the other with less or no effort. Remarkably, even when identical or larger food rewards were available without effort, piglets showed a preference for the compartment previously associated with hidden food, suggesting an intrinsic reward from performing natural foraging behaviors, such as rooting or searching. Notably, all piglets were tested alone, not in social groups, which ensured that choices reflected individual motivational states and not social dynamics.

Social dynamics during feeding may include a subset of contrafreeloading behaviors, as pen mates could choose to work for the food or eat the food after their pen mate did all of the work in a Skinner box-type feeding test. Contrafreeloading has been explored in avian species, offering comparative insights into how domestication and environmental factors shape this

behavior. In Lindqvist and Jensen's (2008) work with *Gallus gallus* (Red Junglefowl) and domesticated *White Leghorn* hens, birds were exposed to an operant task requiring them to peck a key to access food at an early and mature age. Red Junglefowl displayed a greater inclination to work for food compared to domesticated White Leghorns, particularly at a younger age and when doing the test individually. Both breeds showed significantly greater contrafreeloading as juveniles compared to when they reached sexual maturity. When tested individually as adults, no significant difference emerged between breeds. However, when tested in pairs, Red Junglefowl again demonstrated a stronger preference for contrafreeloading than Leghorns, suggesting that social conditions and domestication history shape the expression of contrafreeloading. Ossabaw pigs in many research projects are single housed, which may reduce opportunities for social facilitation of contrafreeloading (NRC, 2011). Their feral origin may nonetheless preserve strong foraging motivation, particularly in settings that permit natural behaviors (Brisbin et al., 2009).

Further evidence of contrafreeloading in poultry comes from a study by Höhne et al. (2023), which investigated foraging motivation in four genetically distinct lines of laying hens housed in group pens. Using a contrafreeloading test modeled after Schütz and Jensen (2001), hens were given a choice between freely available commercial feed and the same feed mixed with wood shavings (1:3 ratio), requiring additional effort to access. Despite having constant access to free food, hens from all lines showed a willingness to work for the mixed feed, with the extent of contrafreeloading varying by genetic line and age. Interestingly, lower-egg-producing lines exhibited a higher proportion of contrafreeloading behavior compared to high-producing counterparts, particularly during early laying stages. This pattern suggests that strong selection for production traits can diminish contrafreeloading motivation. Since Ossabaw pigs have

undergone minimal production-oriented selection, they may be less susceptible to such reductions in foraging drive (Brisbin et al., 2009).

More recently, Van Zeeland et al. (2023) extended the exploration of contrafreeloading behavior to African Grey Parrots (*Psittacus erithacus*), an animal that retains wildtype behaviors, by comparing individuals with and without feather-damaging behavior (FDB), which is a stereotypy that can become a pathological behavior. Parrots were simultaneously offered free food in a bowl and identical food in a foraging device requiring effort to access. Although all parrots engaged in contrafreeloading to some degree, those with FDB spent less time foraging and consumed less food from the foraging device than their healthy counterparts. While the intrinsic drive to forage remains present, chronic behavioral disorders like feather damaging may diminish contrafreeloading behavior, potentially due to altered motivation or disrupted reward processing. While excessive oral stereotypies in commercial pigs are observed when they are confined (Lawrence and Terlouw, 1993; Radkowska et al., 2020), abnormal behaviors among Ossabaw pigs have not been reported.

Nonetheless, contrafreeloading has been studied in commercial swine breeds. Young and Lawrence (2003) investigated contrafreeloading using an operant lever-pressing task in 6 female adult Large White x Landrace crossbreed pigs. Animals housed individually were given continuous access to both free food and an operant panel delivering the same feed. Over extended 120-hour testing periods, pigs demonstrated negligible contrafreeloading behavior; on average, less than 5% of their food intake was earned via the operant device. It is possible that individual housing reduced opportunities for social facilitation, a factor known to influence motivation in pigs, and may have contributed to the limited engagement in contrafreeloading observed (Pedersen et al., 2002). In a social setting, competition or the presence of conspecifics

could increase exploratory and foraging behaviors, potentially resulting in higher engagement with the operant task (Bornett et al., 2000; Boumans et al., 2018).

Inglis et al. (1997) conducted a comprehensive review for understanding contrafreeloading across species by developing a fuzzy logic model that considers multiple interacting factors, such as effort, sensory cues, and individual state. The review emphasized that animals are more likely to contrafreeload when the task offers a moderate challenge and sensory engagement, aligning with natural foraging behaviors. Crucially, they discussed the role of social dynamics, proposing that subordinate individuals may exhibit higher levels of contrafreeloading as a strategy to avoid aggressive encounters at freely available food sources. In social settings where direct competition is costly, working for food may provide a less risky alternative (Inglis, 2022).

From an applied standpoint, contrafreeloading also has major ramifications for obesity treatments, animal welfare, and enrichment design. Encouragement of pigs to participate in deliberate feeding activities, such as pressing paddles or solving basic forage puzzles, not only promotes natural behavioral repertoire but may also assist control intake and enhance metabolic health. Encouraging slower eating, more activity, and cognitive engagement, including contrafreeloading tasks into feeding schedules, could present a fresh approach for controlling obesity in pigs. Furthermore, in long-term research evaluating the effectiveness of environmental or nutritional interventions in pig obesity models, the capacity or inclination to contrafreeload could be a valuable behavioral indicator.

Behavioral Dynamics and Cognition

Obesity and the subsequent weight-loss period not only alter physiological state but also reshape social dynamics and cognitive engagement in pigs. Pair housing with paddle-operated

feeders, which is the case of the current study, creates a socially competitive environment in which feeding became a domain of dominance negotiation, task performance, and behavioral flexibility (Bornett et al., 2000; D'Eath, 2005; Street and Gonyou, 2008; Ede and Parsons, 2023).

Contest measures provide a valuable paradigm wherein animals evaluate opponents and resource worth according to their own resource-holding potential, perceived rewards, and information acquired during interactions (Parker, 1974; Arnott and Elwood, 2009). Certain pigs may opt to participate in short contests, while others may either continue or withdraw, regardless of variations in body size, which underscores the impact of cognitive assessment above simply physical advantages (Mendl et al., 2009). A recent study by Lee et al. (2024) indicated that pigs that could alter their learned associations were far more likely to win dyadic contests. This finding suggests that being able to adjust and quickly update information can make up for physical advantages. These results go against the idea that size alone determines who wins, and instead show that intellect plays a changing role in the outcome of contests (Oldham et al., 2025).

As contests get more intense, behavior becomes more complicated. Camerlink et al. (2016) looked at pig contests and broke them down into four levels of escalation, from displays to full fights. They found that aggressiveness and past experiences could predict whether an interaction would continue or end early. When a pig was able to accurately judge the balance of advantage, escalations often stopped. This supports the idea of cognitive appraisal in social conflict. Social network analysis has also shown how cognitive traits show up in the way groups are set up. Automated monitoring systems show that pigs form hierarchies based on centrality measures like betweenness and eigenvector scores, which are linked to their roles in aggression and getting resources (Oldham et al., 2025). Pigs that were more central were more likely to

engage in selective behavior or withdraw strategically. These behaviors suggest that they were making informed social decisions (Camerlink et al., 2016; Foister et al., 2018).

Anticipation is another way that cognition helps contest strategies. Pigs become selectively aggressive toward competitors if it is likely less costly to engage in combative aggression. For example, researchers suggest that pigs likely “size their opponent up” before deciding if they will use a combative strategy or a non-combative strategy to engage in a contest. Animals with cognitive foresight can tell the difference between opponents by valuing contests that give them a clear edge and staying away from expensive ones (Arnott and Elwood, 2009; Camerlink et al., 2015; Camerlink et al., 2017). Learning how to use the feeder mechanism added even more mental challenges to social interaction. Individuals who learned faster or had better cognitive skills probably gained feed more reliably and stayed out of expensive fights. On the other hand, pigs that do not respond well to social defeats or exclusion can become more stressed (Ruis et al., 2001; Toyoda, 2017; Lee et al., 2024; Oldham, 2024).

The interactions among decision-making, contrafreeloading, and competition strategies behavior potentially improve translational research. Feeders that require operant conditioned tasks, such as paddle pressing, are one example of an environment that allows for the measurement of social competition, contrafreeloading, and cognitive engagement. Such environments may help individuals become more socially resilient, less aggressive, and have better welfare outcomes (Raoult et al., 2024).

Conclusion

Rising from a complex interaction of biological, behavioral, social, and financial elements rather than only from caloric imbalance, obesity remains one of the most urgent public health issues of the twenty-first century (Swinburn et al., 2011). The obesity development starts

with food availability, proceeds through physiological control and behavioral reactions, and shows up at individual, group, and species levels (Berthoud, 2012; Brisbois et al., 2012). Accurate measurement methods and physiological markers are only one aspect of understanding the variety in how obesity develops and evolves; another is a thorough awareness of the social constructions around body image, food choices, and newly developing pharmacological therapies, including GLP-1 agonists (Rubino et al., 2016). In this setting, animal models, especially the Ossabaw pig, because of their special evolutionary and epigenetic features, offer a vital forum for exploring the metabolic and behavioral aspects of early-stage obesity (Matthan et al., 2018). Technologies such as ultrasound offer easily available, non-invasive techniques for body composition monitoring in such models, hence improving the accuracy of longitudinal studies (Wagner, 2013). Offering dietary assistance in settings of food insecurity and metabolic disturbance, sorghum kombucha also shows promise as an intersecting nutritional value, polyphenolic bioactivity, and sustainability (Lopes et al., 2019). At last, including behavioral insights, such as contrafreeloading, social interactions, and motivational dynamics, into these models enhances our knowledge of the lived experience of obesity and increases its translational relevance to human health (Yu et al., 2022; Satyal et al., 2021; Spyridaki et al., 2016; Inglis, 2022).

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Tables

Table 1.1 Overview of obesity progression stages, highlighting metabolic alterations, physiological responses, and clinical manifestations associated with pre-pathological, pathological responses and chronic phases of obesity-related metabolic disease.

Stage	Category	Description	Key Metabolic Features	Clinical Signs
Early Obesity	Pre-Pathological/ Preclinical	Body fat begins to accumulate due to excess energy intake; metabolic flexibility still intact	- Mild insulin resistance - Subclinical inflammation - Adipocyte hypertrophy (enlargement)	- Often asymptomatic - May show mild weight gain
Metabolic Dysregulation	Pre-Pathological/ Subclinical	Adipose tissue expands beyond capacity; spillover to ectopic sites begins	- Increased circulating free fatty acids - Elevated fasting insulin - Beginning hepatic steatosis (fatty liver)	- Possible fatigue - Increased waist circumference - Elevated fasting glucose or insulin (but still within normal limits)
Metabolic Syndrome	Pathological/ Clinical	Obesity triggers metabolic disturbances that meet criteria for disease	- Insulin resistance - Hyperlipidemia ($\uparrow$ LDL, $\downarrow$ HDL) - Elevated blood pressure - Systemic inflammation	- Diagnosed metabolic syndrome - High blood pressure - Impaired glucose tolerance
Chronic Obesity	Pathological/ Chronic	Long-term exposure to metabolic stress leads to irreversible damage	- Type 2 diabetes mellitus (T2DM) - Non-alcoholic steatohepatitis (NASH) - Cardiovascular disease (CVD)	- Diagnosed T2DM, NASH, or CVD - Medically managed obesity - Organ damage
Morbid Obesity	Advanced Pathological/ Chronic	Obesity results in multi-organ complications and high disease burden	- Chronic systemic inflammation - Sarcopenic obesity - End-organ failure	- Mobility limitations - Multiple comorbidities - Reduced quality of life

Adapted from Eckel et al., 2005; Gregor and Hotamisligil, 2011; Engin, 2017; Blüher, 2019

Chapter 2 - Sorghum Kombucha Influences Average Daily Gain and Percentage of Back Fat Loss in Obese Ossabaw Pigs During Early Weight Loss

Abstract

The objective of this study was to determine if sorghum kombucha can be used to modify body composition, reduce body weight (BW) gain, and back fat (BF) gain in lean or obese Ossabaw pigs (Corvus Biomedical, IN; n = 32; gilts and boars; pair housed; sex and treatment balanced) undergoing a structured weight loss program. At 3.3 ± 0.52 months of age, pigs were randomly assigned to either a lean (LN) diet (5L80, Purina) or an obesogenic Western (OBWL) diet (KT324, Corvus) for 16 weeks, and to receive either an isocaloric solution (ISO) or fermented sorghum kombucha (SK) for 6 weeks (USDA-ARS, Manhattan, KS). Following the diet induction phase, obese pigs were transitioned to a reduced-calorie diet (OBWL). Ultrasound imaging was used to assess back fat thickness (BF) and loin eye area (LEA), and body weight (BW) was recorded twice weekly. During the post-intervention period, OBWL provided fermented sorghum kombucha had significantly lower average daily gain (ADG; $P = 0.005$) compared to all other pigs. A linear effect ($P < 0.001$) was observed for BW% change ($10.7, 10.0, 3.8, \text{ and } 1.6 \pm 1.58 \text{ SEM } \%$ for LN-ISO, LN-SK, OBWL-ISO, and OBWL-SK, respectively). Although all OBWL pigs had greater ($P = 0.013$) BF thickness than all LN pigs ($0.94 \text{ vs. } 1.63 \pm 0.104 \text{ SEM cm}$) during the 2nd phase (last 6 weeks), there was a linear effect ($P = 0.002$) for % change in BF among the 4 treatments ($19.7, 7.9, -0.4, \text{ and } -10.8 \pm 5.42 \text{ SEM } \%$ for LN-ISO, LN-SK, OBWL-ISO, and OBWL-SK, respectively). OBWL-SK pigs had a decrease (loss LEA) in LEA% change compared to LN-SK pigs that gained LEA (Time*Body-

Type*Solution $P = 0.034$). Sorghum Kombucha may improve BF loss and decrease ADG during weight loss in obese subjects. The shrinkage of LEA in the early weeks of weight loss may be due to depletion of glycogen, which causes increased water loss. More research is needed with a longer duration of a Sorghum Kombucha treatment and weight loss period to evaluate the long-term effects and underlying mechanisms of this intervention.

Keywords: Sorghum Kombucha; Polyphenols; Ossabaw pigs; Obesity; Ultrasound; Swine

Introduction

Obesity is increasingly recognized as a chronic, low-grade inflammatory condition that may impact brain function, metabolic health, body composition, and overall well-being in both humans and domestic animals (Calder et al., 2017; Bennett et al., 2018; Stefanaki et al., 2018; Hofseth and Hébert, 2022). Characterized by an overaccumulation of fat in hypertrophic adipocytes, obesity is associated with elevated levels of pro-inflammatory cytokines, which contribute to the development of metabolic syndrome, insulin resistance, and neurological disorders (Elenkov et al., 2005; Balistreri et al., 2010; Klötting and Blüher, 2014; Torres et al., 2019; De A. Boleti et al., 2023). Paradoxically, obesity is increasingly observed in populations experiencing food insecurity, a phenomenon known as the food insecurity–obesity paradox. In the United States, approximately one in seven households is food insecure (USDA, 2023), yet individuals in these households face a 32% higher risk of obesity, with an even more pronounced effect among women and girls, who face up to a 50% increased risk (FRAC, 2010; Seligman et al., 2010). In these contexts, limited access to affordable, nutrient-dense food often leads to overconsumption of calorie-dense, nutrient-poor diets, particularly those high in fat, sugar, and processed ingredients. This type of dietary pattern contributes to systemic inflammation and

metabolic dysfunction, despite the presence of moderate food scarcity (Drewnowski and Specter, 2004; Seligman et al., 2010; Franklin et al., 2012).

Given the complexity of obesity and its multiple causes, dietary interventions that can reduce inflammation and poor body composition connected with a Western-style diet are generating increasing interest (Popkin and Gordon-Larsen, 2004). One such intervention involves polyphenol-rich plant compounds, which have been shown to reduce oxidative stress and inflammation (Manach et al., 2004; Scalbert et al., 2005; Pandey and Rizvi, 2009). Among promising sources of polyphenols is sorghum, a drought-tolerant cereal crop in which the United States is the world's leading producer (USDA ERS., 2022; USDA, 2023). Despite this, less than 2% of sorghum is currently processed for human consumption. Dark-colored sorghum varieties (e.g., red or black) are especially rich in polyphenols and possess strong antioxidant and anti-inflammatory properties (Awika, 2017; Singh et al., 2024).

Sorghum can be used to produce kombucha, a fermented, probiotic-rich beverage traditionally made from tea. When seeped-sorghum is used as the base, the resulting beverage retains high levels of bioactive polyphenols while offering the added benefit of fermentation-enhanced bioavailability. Emerging evidence suggests that kombucha produced from sorghum may offer a novel and accessible strategy to improve body composition and reduce inflammatory responses associated with diet-induced obesity (Carnahan et al., 2014; I. Mohamed et al., 2022).

The Ossabaw pig is widely regarded as the most relevant large animal model for studying human obesity and related metabolic disorders (Conn, 2013; Newell-Fugate et al., 2015; Matthan et al., 2018). These pigs carry a "thrifty genotype" that enables rapid fat accumulation in response to environmental and dietary stressors. When fed a Western-style diet, Ossabaw pigs develop hallmark features of human metabolic syndrome, including insulin resistance,

dyslipidemia, and increased visceral adiposity (Lee et al., 2009; Toedebusch et al., 2014; Sturek et al., 2020). Their physiological similarity to humans in terms of adipose distribution, inflammatory responses, and cardiovascular function makes them ideal for translational research (Zhang et al., 2021).

To monitor body composition in a non-invasive manner, ultrasound imaging is frequently used in animal studies. Ultrasound imaging provides accurate measurement of back fat (BF) thickness and loin eye area (LEA), which may provide more detailed information about adiposity and lean tissue distribution than body weight alone (Szabo et al., 1999; Vargas Junior et al., 2021; Basak et al., 2023). Therefore, ultrasound is an essential tool for evaluating the outcomes of nutritional interventions aimed at modulating fat accumulation and muscle preservation over time (Cutshaw et al., 2014; Prado and Heymsfield, 2014).

The primary objective of this study was to evaluate whether sorghum kombucha could influence changes in bodyweight, BF, and LEA in lean pigs or obese pigs that changed from a hypercaloric Western-style diet to a calorie-appropriate normal diet. We hypothesized that pigs receiving sorghum kombucha would have decreased back fat accumulation and average daily gain and a lower LEA reduction compared to isocaloric-treated pigs, thereby supporting the potential role of fermented sorghum beverages as a functional nutritional supplementation to a weight loss program.

Materials and Methods

Animals, Housing, and Husbandry

All experimental procedures followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching and were approved by the Institutional Animal Care and Use Committee (IACUC #4831). A total of 32 Ossabaw pigs (16 gilts and 16 boars; Corvus

Biomedical, LLC, Crawfordsville, IN) were utilized in a two-block design, with 16 pigs per block. One block was conducted between April and October of 2023, and the second block was conducted between January and July of 2024. Pigs remained at Corvus Biomedical (Crawfordsville, IN) for the first phase of the experiment and were later (6 ± 0.52 months of age) transported for 8 hours to the Large Animal Research Center (LARC) at Kansas State University (Manhattan, KS), to support structured data collection. Upon arrival, animals were housed in climate-controlled rooms with 3.7 x 3.7 m pens, each equipped with a 1.6 x 1.2 m automated feeding station. Each pair was assigned to pens in a way that ensured treatment and sex were balanced across both sides of the housing room (4 pens per side). Pigs had ad libitum access to water and received feed twice daily. Pigs were provided dynamic environmental enrichment items, including Astros and Lunas (EasyFix, SD, USA), fixed bells, and milk jugs, and static environmental enrichment such as fixed chains and a scratch-mat pole.

Treatment Assignments

The sample size for each treatment was determined (Martin et al., 1987) using body weight variances from obese adult male and female minipigs before and after weight loss interventions of either bariatric weight loss surgery or reduced feed (Gautier et al., 2020). Calculations were based on 4% of the desired minimum detectable difference from the control. A significance level (α) of 0.05 and a statistical power ($1-\beta$) of 0.95 were applied, which resulted in up to 4 animals per treatment (4 treatments). For sex (boar and gilt) to be balanced through the experiment, 8 animals per treatment were used.

Before diet treatments were initiated, all pigs were maintained on a standard grower feed (DuMOR Hog Grower; Purina, Richmond, IN). Animals were enrolled at 3.3 ± 0.52 months of age, when all treatments were assigned. To safeguard against imbalanced body weights and

competition within a pair, pigs in the top and bottom quartile body weights were first weight and sex matched. Pigs with intermediate body weights were then randomized into sex-matched pairs using a random number generator in Excel. Then, each pair was randomly assigned to lean or obese body types (Lean, LN; Obese, OBWL) and then randomized into receiving a liquid supplementation in the last 6 weeks of the experiment (isocaloric controls, ISO; Sorghum Kombucha, SK). Lean pigs received a calorie-appropriate chow throughout the entire duration of the study (LabDiet Mini-Pig HF Grower 5L80; Purina Test Diet, Richmond, IN). Obese pigs were stepped up to a hypercaloric, high-fat, Western diet (MetS; KT324; Corvus Biomedical, IN) for 16 weeks, then they were stepped down from the Western diet and fed the same calories and diet as lean for the last 6 weeks of the experiment (Figure 2.1).

The normal diet, providing up to 1,650 kcal/day, was designed to support healthy development and consisted of 9.9% fat (ether extract), 70.3% carbohydrates, and 19.7% protein. In contrast, the Western diet delivered up to 3,400 kcal/day and was formulated to induce obesity, with 40.8% of carbohydrate-derived calories from high-fructose corn syrup, 42.9% of the dietary energy from fat is attributable to components classified as ether extract (traditional lipids like triglycerides from oils or animal fat), based on chemical analysis, and 16.9% protein.

To reduce gastrointestinal discomfort during dietary changes, step-up and step-down strategies were implemented using 25% changes for 3 days (25, 50, 75% change for 3 days for each change). Lean pigs underwent a 9-day step-up from their original standard grower diet (DuMOR Hog Grower; Purina, Richmond, IN) to the LabDiet, then remained on the diet for the entire experiment (Figure 2.1). Pigs in the obese group were step-up transitioned to the Western diet (MetS; KT324; Corvus Biomedical, IN) after enrollment, and then stepped down after 16 weeks, prior to liquid supplementation (Figure 2.1).

Liquid supplementation was applied for the last 6 weeks of the study (Figure 2.1). Both lean pigs and obese pigs on the weight loss program were administered twice daily (500 mL after each meal) either an isocaloric solution (ISO; 0.03 calories/mL from sucrose) or fermented sorghum kombucha (SK; 0.03 calories/mL after microbial processing of nutrients). Supplementation was provided 3-5 minutes after the feed was released in the bowls adjacent to the feed bowls. Both solutions were developed and tested previously (Nelson et al., 2023) and provided each by the United States Department of Agriculture – Agricultural Research Service (USDA-ARS, Manhattan, KS). Both treatments provided an energy value of 0.03 kcal/mL.

Daily and Weekly Routines

Throughout the study, pigs followed a consistent weekly routine to support data collection and minimize handling-related stress. Every day, feeding tests were implemented at 0800 and 1600 h, fecal matter was removed, and after feeding, feeders were cleaned. On Mondays, Tuesdays, and Wednesdays, in the mornings prior to feeding, pigs were involved in an 8-arm radial maze individual task as part of the broader research project (Scott, 2025). On Mondays, pigs were weighed before completing the maze task. Each Thursday, prior to morning feedings, pigs were weighed and then immediately directed into a containment sling (Panepinto, Loveland, CO), a low-stress restraint technique first developed in the 1960s for humane swine handling. The sling was used to safely immobilize the animals in a consistent position, allowing for efficient ultrasound image acquisition while minimizing physical restraint and associated stress. In between feedings on Thursdays, pigs were placed in pairs in holding pens while pens and feeders were sanitized. On Fridays, Saturdays, and Sundays, only daily tasks (feeding, liquid supplementation) occurred in the mornings and evenings.

Body Weight, Ultrasound, and Handling

Body weight was recorded at birth, then measured weekly starting at 3.3 ± 0.52 SD months of age and increased twice weekly after 5.3 ± 0.52 SD months. Weights were obtained using an Avery Weigh-Tronix Scale (Fairmont, MN) installed in a Mini Swine Transfer/Restraint Cage (Animal Care Products, Inc., College Station, TX). Pigs were individually guided from their home enclosures to the scale using hog boards. After they were weighed, the pigs were moved to the sling for handling procedures.

As previously described by Scott (2025), a positive reinforcement, a fruity-flavored tiny marshmallow (Jet-Puffed Fruity-Fun Mini Marshmallows, Kraft Heinz; Davenport, IA), was given once the pig's legs were in the sling's appropriate cut-outs, but only when the pig behaved calmly. The sling was gently raised with a hand crank once the pig relaxed. The technicians gently touched the pig during the suspended period to help reinforce comfort and reduce stress. After collecting the data, the sling was softly lowered, and the pig was returned to its pen using hog boards. This training procedure was carried out up to once weekly, both at Corvus Biomedical and at LARC, to promote familiarity and minimize handling stress during future procedures.

Once the pig was stable and calm in the sling, ultrasound imaging was conducted weekly beginning at 6.3 ± 0.52 SD months. Two different ultrasound devices were used across experimental blocks: The M-turbo (FUJIFILM SonoSite, Bothell, WA) in Block 1 (2023), and the Butterfly iQ+ (Butterfly Network, Burlington, MA) in Block 2 (2024). The M-Turbo uses traditional piezoelectric crystal transducers, while the Butterfly iQ+ employs capacitive micromachined ultrasound transducer (CMUT) technology, allowing a single probe to perform multiple imaging functions. On both ultrasounds, imaging was performed using the

musculoskeletal preset, with a gain setting of 50% and 6× magnification. The probe was positioned perpendicular to the pig's back, just below the spine, at the level of the loin eye area, located between the last two ribs. A 70% isopropyl alcohol antiseptic spray (Equate, Calabasas, CA) was applied to the imaging site to improve probe contact and image clarity. In Block 1, pigs were shaved using a standard electric razor to ensure optimal image quality. Shaving was not required in Block 2 due to differences in probe sensitivity and design between the two ultrasound systems.

Ultrasound Images

Ultrasound images from both experimental blocks were analyzed using the ImageJ software (version 1.53t, National Institutes of Health, Bethesda, MD). ImageJ is an open-source image processing and analysis program developed in Java (Schneider et al., 2012). Prior to analysis, all images were converted to 8-bit format, and the scale was calibrated for each image using the standard ultrasound scale bar located on the left side of the image. Back fat (BF) thickness was measured in centimeters (cm) using the straight-line tool, while loin eye area (LEA) was measured in square centimeters (cm²) using the polygon selection tool. The same trained technician performed all ultrasound imaging and conducted all measurements in ImageJ.

Statistical Analysis

All statistical analyses for body weight and ultrasound-derived outcomes were conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC). Data distributions were evaluated for normality using the Shapiro-Wilk test within the UNIVARIATE procedure. Due to the distinct experimental phases, statistical analyses were performed separately for the 16-week Western diet induction period and the subsequent 6-week weight loss and liquid supplementation phase. The separation was necessary because pigs in the OBWL groups underwent a dietary shift

at Week 16, introducing a new biological condition. Each dataset was analyzed using the same modeling structure described above, with fixed effects, repeated measures, and random effects appropriately specified for each phase.

For repeated measures analyses, covariance structures evaluated included compound symmetry (CS), first-order autoregressive [AR(1)], and heterogeneous autoregressive [ARH(1)]. The optimal structure was selected based on the Akaike Information Criterion (AIC) or based on convergence criteria when AIC values were not obtainable. Models were estimated using residual maximum likelihood (REML), and the degrees of freedom method was the Satterthwaite approximation to account for pen-level variability. The experimental unit was the pen, with two pigs (sex- and weight-matched) per pen randomly assigned to one of four treatment groups. The pen was specified as the subject effect, with repeated measures defined by week or session nested within week, depending on the outcome variable. Fixed effects included treatment, block, week, and their interactions. Block and covariates, birth weight, and sex (boar or gilt) were retained in the model only if $P \leq 0.10$. Pen nested within treatment was included as a random effect. Pairwise comparisons (Tukey-Kramer adjustment) were conducted using two approaches based on the study objectives: (1) among treatments at each time point using a sliced-effect multiple comparison approach, to evaluate differences in body weight and composition at specific weeks, and (2) within each treatment across time to assess longitudinal responses and treatment-specific progression. Additionally, pre-planned orthogonal polynomial contrasts were used to test for linear and quadratic trends across biologically relevant treatment orders (Order of LN-ISO vs. LN-SK, OBWL-ISO vs. OBWL-SK) to evaluate potential directional effects such as progressive reductions in body weight. Statistical significance was declared at $P < 0.05$, while

tendencies were interpreted for *P*-values between 0.05 and 0.10 when supported by biological relevance.

Results

Prior to liquid supplementation, obese pigs exhibited a greater acceleration of body weight gain compared to lean pigs, starting at just 2 weeks after the western diet was applied, then ending with a 13.4 kg difference at the end of 16 weeks (TRT*Time, $P \leq 0.001$; Figure 2.2). Body weight change (percent difference from pre-supplementation weights) among all lean pigs was greater than all obese pigs from weeks 2 to 6 relative to liquid supplementation (TRT*Time, $P \leq 0.001$; Figure 2.3). A significant treatment effect was observed for average daily gain (ADG) during the liquid supplementation phase (TRT, $P \leq 0.001$; Figure 2.4A). The least average daily gain was observed in OBWL-SK pigs (TRT, $P \leq 0.001$; Figure 2.4A). The linear trend for both ADG and body weight % change was significant (Linear, $P \leq 0.001$; Figure 2.4).

As expected, all lean pigs had less total back fat thickness compared to all obese pigs (TRT, $P \leq 0.001$; Figure 2.5A), but linear significance was observed for percentage change in back fat (%BF) thickness (Linear $P = 0.002$; Figure 2.5B). Lean pigs supplemented with sorghum kombucha had a slower BF gain than isocaloric pigs (Figure 2.5B). There was minimal gain and some backfat loss among OBWL pigs supplemented with isocaloric solution, but all OBWL pigs supplemented with sorghum kombucha had back fat loss (Figure 2.5B).

Throughout supplementation, the OBWL pigs had greater total loin eye area than LN pigs (TRT, $P \leq 0.001$; Figure 2.6), and a significant, albeit less prominent, sloped linear trend was observed ($P = 0.026$; Figure 2.6B) with some minor losses of LEA size in OBWL pigs.

Discussion

To the best of current knowledge, this is the first study evaluating, in any other animal model or in Ossabaw pigs, the effect of sorghum kombucha supplementation on weight loss-related outcomes (Naiel et al., 2025; Scott, 2025). Although the literature on sorghum kombucha is limited, some studies have investigated its fermentation processes and potential health-related properties. Galdamez (2023) explored fermentation across three whole-grain sorghum varieties (White, Waxy, and Sumac) and observed that while all exhibited significant acidification during a 7-day kombucha process, the Sumac variety produced the highest phenolic content, suggesting superior antioxidant potential. Kiptanui et al. (2022) evaluated the fermentation of kombucha using malted and unmalted sorghum with three different SCOBY (Symbiotic Culture of Bacteria and Yeast) types, demonstrating that malted sorghum fermented with SCOBY from mixed culture from hibiscus kombucha produced the highest phenolic content and antioxidant capacity by the end of the 7-day fermentation. Collectively, these studies focused on physicochemical and microbiological properties, providing foundational evidence that sorghum kombucha may serve as a functional beverage with antioxidant, antimicrobial, and probiotic potential.

While studies on the effects of sorghum-based kombucha in animal models are still lacking, several investigations have explored the impact of tea-based kombucha, commonly available on the market, primarily using rodent models (Hartmann et al., 2000; Morshedi and Dashti-Rahmatabadi, 2010; Costa et al., 2022; Moreira et al., 2022), with a few extending to human participants (Fraiz et al., 2024). In a mouse model of diet-induced obesity progressing to nonalcoholic fatty liver disease (NAFLD), Moreira et al. (2022) administered tea kombucha to one group while the control group received no supplementation. Mice that consumed kombucha exhibited improved glucose tolerance, reduced liver fat accumulation, and downregulation of

pro-inflammatory and lipogenesis-related gene expression, including TNF- α , compared to the non-supplemented group. In a 10-week randomized controlled trial among human individuals with excess body weight, Fraiz et al. (2024) found that daily consumption of green tea kombucha (200 ml) alongside caloric restriction significantly reduced cardiometabolic risk (LAP), stabilized IL-6 concentration compared to the control group, and improved salivary microbiota diversity, suggesting anti-inflammatory and microbial benefits. These findings align with those of the current study, in which OBWL pigs supplemented with sorghum kombucha demonstrated the lowest average daily gain and a reduction in back fat, suggesting that sorghum kombucha may promote fat mobilization during weight loss.

Given that the individual bioactive compounds present in kombucha, particularly polyphenols, which are known for their antioxidant and anti-inflammatory properties (Jayabalan et al., 2014), have demonstrated metabolic benefits in other species, no published studies to date have evaluated the impact of sorghum kombucha on body weight, subcutaneous fat, or loin muscle area in pigs. However, Nelson et al. (2023) investigated behavioral responses to sorghum kombucha in lean adult Ossabaw pigs using a three-arm maze preference test and found that pigs consistently preferred the sorghum kombucha solution over an isocaloric control. Thus, the present study fills a critical gap in the literature and offers foundational insight into the potential agrimedical significance of sorghum-based fermented beverages.

Previous studies in Ossabaw pigs have demonstrated that the extended exposure to obesogenic western diets (24 to 40 weeks) can induce metabolic syndrome, including the development of full-blown metabolic syndrome characterized by obesity, glucose intolerance, hyperinsulinemia, and hypertension (Dyson et al., 2006). More critically, Ossabaw pigs in previous research exhibited more extensive native coronary artery disease, greater in-stent

restenosis, and impaired coronary perfusion compared to Yucatan pigs (Neeb et al., 2010). The pigs enrolled in the present study were not fed to the point of severe or morbid obesity. Instead, the experimental design intentionally avoided inducing severe obesity stages, such as those where animals become non-ambulatory or develop debilitating comorbidities. The rationale was based on a translation perspective, rather than evaluating sorghum kombucha as a curative agent for obesity, the goal was to examine its potential as a supportive nutritional tool during the early stages of an early weight loss process. The model better reflects individuals who are still metabolically responsive and physically active. Based on circulating blood markers reported by Scott (2025) of the same pig cohort, pigs displayed elevated, but not extreme, levels of glucose, insulin, and cholesterol, consistent with early insulin resistance and pre-diabetic profiles (preclinical stage). The parameters support the notion that the pigs were in a transitional metabolic state, where dietary interventions such as sorghum kombucha could have meaningful physiological effects.

During the 16 weeks on a Western diet, Ossabaw pigs in the obese group exhibited a consistent and progressive increase in body weight. Such a pattern is consistent with the well-established metabolic phenotype of the Ossabaw breed, which is genetically predisposed to excessive weight gain when exposed to energy-dense diets. In previous studies, Ossabaw pigs fed a Western-style diet gained up to 40% of their baseline weight within a 10-week period, accompanied by increased adiposity and metabolic alterations characteristic of early-stage obesity (Lim et al., 2018). In a long-term study conducted by Gautier et al. (2020), Yucatan minipigs in a western diet reached a weight 60.7% higher than the expected growth curve after 32 weeks of feeding a western diet, which was 16 weeks longer than pigs in the current experiment.

Having completed 16 weeks with a Western diet followed by a weight loss program and kombucha supplementation for 6 weeks, Ossabaw pigs in the current experiment displayed clear temporal patterns in body weight and average daily gain. Those in the OBWL-SK group exhibited the least average daily gain during the 6 weeks of the weight loss program. Reinforcing the idea that sorghum kombucha attenuates weight gain in obese subjects even under reduced-calorie regimens. Introducing sorghum kombucha appears to slow weight accumulation beyond what would be expected from reduced-calorie intake alone. The observed metabolic response extends previous findings with clinical evidence from a 10-week randomized controlled trial, where participants (humans) consuming green-tea-based kombucha during energy restriction showed greater improvements in lipid metabolism and gastrointestinal symptoms compared to those on diet alone (Fraiz et al., 2024). For instance, in a study using high-fat diet-induced obese rats, extruded sorghum flour significantly reduced body weight and adiposity, likely via modulation of inflammation and metabolic signaling pathways (Arbex et al., 2018). Although that study involved extruded rather than fermented sorghum, both processes may enhance the bioavailability of bioactive compounds such as polyphenols, which play a critical role in energy regulation and fat metabolism.

Interestingly, while sorghum kombucha improved the reduced weight gain in Ossabaw pigs under a calorie-restricted regimen, a contrasting pattern is observed in diabetic rodent models consuming tea kombucha. For instance, Morshedi and Dashti-Rahmatabadi (2010) found that diabetic male wild-type Wistar rats (chemically induced diabetic models) receiving chronic kombucha tea supplementation (prepared from black tea) experienced a reversal of diabetes-induced weight loss, maintaining their initial body weight throughout the 15-day intervention, while the diabetic control group that did not receive the kombucha continued to lose weight

significantly. Similarly, Mallmann et al. (2022) reviewed that green tea kombucha significantly prevented body weight reduction in wild-type diabetic Wistar rats compared to untreated controls, suggesting potential anabolic or anti-catabolic effects in metabolically compromised rodents. However, these effects appear to be model-dependent or influenced by the stage of diabetic progression. While diabetic rodents show preservation or gain of body mass when treated with tea-based kombucha, likely due to attenuation of hyperglycemia-induced catabolism, the pigs in this study were not diabetic but rather obese-prone and fed a high-fat Western diet. Thus, the metabolic context may dictate whether kombucha results in weight preservation or weight suppression.

Back fat thickness is a key indicator of subcutaneous adiposity and is highly responsive to dietary and metabolic interventions. In the current study, obese Ossabaw pigs receiving sorghum kombucha alongside a weight-loss protocol (OBWL-SK) exhibited a notable reduction in back fat percentage compared to obese controls (OBWL-ISO) receiving only the weight-loss diet. Comparable reductions in fat accumulation have been observed in rodent models. For example, Lee et al. (2019) demonstrated that genetically modified diabetic rats receiving black or green tea-based kombucha experienced attenuated hepatic steatosis and reduced liver triglyceride concentrations through mechanisms involving decreased lipid peroxidation. Kombucha supplementation has also been linked to improvements in gut microbial composition. Costa et al. (2022) found that Wistar (wild-type) rats fed a high-fat, high-fructose diet and supplemented with green or black tea kombucha showed reduced abundance of gram-negative bacteria such as *Proteobacteria* and *Bacteroides*, alongside increases in beneficial genera like *Adlercreutzia*.

In vitro research by Wanyo et al. (2025) further highlighted the functional potential of kombucha made from mulberry leaf tea, which demonstrated antioxidant, anti-inflammatory, and

antihyperglycemic properties attributed to enhanced bioaccessibility of phenolic acids such as *p*-coumaric and sinapic acids, as well as the presence of 1-deoxynojirimycin. Taken together, these studies suggest that kombucha derived from various plant substrates may influence lipid metabolism and adipocyte function through polyphenol-driven mechanisms.

Despite the positive impact on back fat reduction, obese pigs in the current experiment, whether supplemented with kombucha or not, still retained significantly more back fat than their lean counterparts after a six-week intervention period. The outcome may show that back fat typically requires a longer weight loss intervention duration. Nonetheless, the greatest backfat percent loss and the least ADG were observed in the obese pigs supplemented with sorghum Kombucha. Although pig persistent backfat underscores the metabolic rigidity of the Ossabaw breeds once they become obese, the individual changes in weight loss and back fat loss may serve as better indicators of early weight loss success. More obese pigs lost back fat when they were supplemented with sorghum kombucha, and the linear trend indicates that sorghum kombucha may even slow back fat accumulation among lean pigs.

There was some LEA loss among obese pigs, and little evidence that the liquid supplementation influences LEA loss among obese pigs in the current study. The early shrinkage of the loin eye area observed during the weight-loss period likely reflects muscle glycogen depletion, which in turn causes water loss. Glycogen stores in muscle can bind 2–4 grams of water per gram of glycogen, so when glycogen is depleted during restricted feeding, hydration, and apparent muscle size diminish (Przybylski et al., 2006). Strategic feeding studies have found that reducing digestible carbohydrates just before slaughter can lower muscle glycogen by 11–26%, correlating with observable shrinkage in loin area (Rosenvold et al., 2001). Consequently, the decline in LEA noted in the obese group in a weight loss program in pigs during the initial

weeks may not indicate actual muscle loss, but rather transient anatomical changes caused by decreased water retention due to rapid glycogen depletion.

Conclusions

Sorghum kombucha supplementation during a weight loss program reduced average daily gain and supported back fat reduction in obese Ossabaw pigs. However, LEA shrinkage in the early weeks of weight loss was similar between the kombucha and control groups, likely reflecting glycogen and associated water depletion rather than a treatment effect. No significant effects were observed in lean pigs. The current findings suggest that fermented sorghum kombucha may be a useful adjunct to weight loss programs for reducing adiposity in metabolically compromised individuals, though longer-term studies are needed to evaluate its impact on muscle preservation and to clarify underlying mechanisms.

Implications

This study adds to the growing evidence that fermented sorghum products may offer functional benefits in managing obesity-related outcomes. By demonstrating that sorghum kombucha can improve the reduction of back fat and slow weight gain in an established model of obesity, this work highlights the potential of fermented, polyphenol-rich grains as part of dietary strategies aimed at mitigating excess adiposity. As food insecurity and obesity increasingly intersect, particularly in underserved communities, affordable and shelf-stable ingredients like sorghum, especially when enhanced through fermentation, may represent accessible nutritional tools. In the context of commercial swine production, managing body composition, particularly reducing excessive back fat, is a key concern for optimizing carcass quality and animal welfare. While fermented functional beverages like kombucha are not yet integrated into standard pig

diets, this research supports the potential for fermented sorghum-based products to be developed into cost-effective feed additives that improve fat metabolism in over-conditioned animals.

Additionally, this study highlights the value of using ultrasound to assess body composition in both animal and human models. In humans, body weight alone is often insufficient to evaluate metabolic health, given the variability in fat distribution and lean mass across individuals. Ultrasound offers a practical, non-invasive method to assess subcutaneous fat and muscle area with greater precision. Its increased application in obesity research could improve phenotyping and intervention monitoring, particularly in populations where traditional measures like BMI fall short (Wagner, 2013; Prado and Heymsfield, 2014).

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Figures

Figure 2.1 Experimental Timeline: Diet Transitions and Liquid Supplementation in Lean and Obese Ossabaw Pigs.

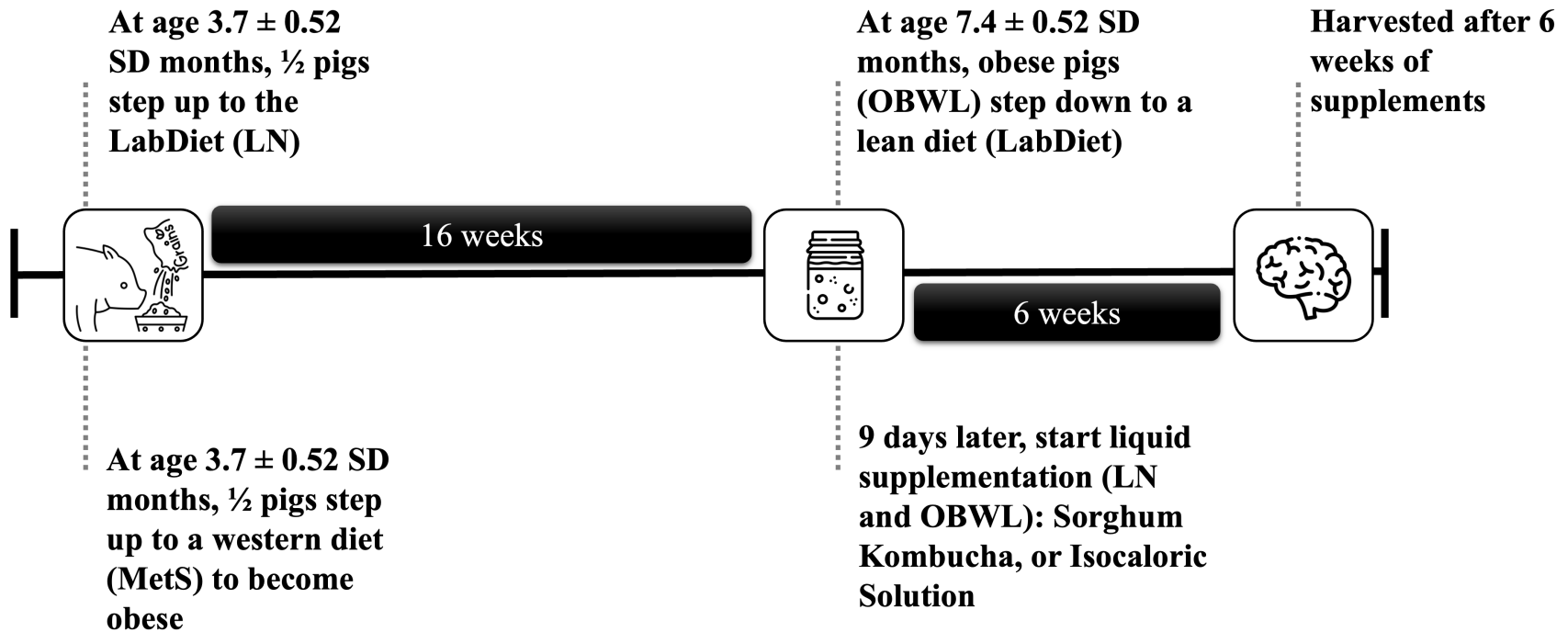


Figure 2.2 Weekly body weight (kg) of lean and obese Ossabaw pigs (n=32, gilts and boars) from baseline (week -3) through week 16, before the weight loss program was applied to the obese group and liquid supplementation was applied to the lean (no diet change) and obese groups (TRT*Time, $P \leq 0.001$). Error bars represent standard error mean (SEM). *LS-Means differed for lean vs. obese pigs (Tukey-Kramer adjustment).

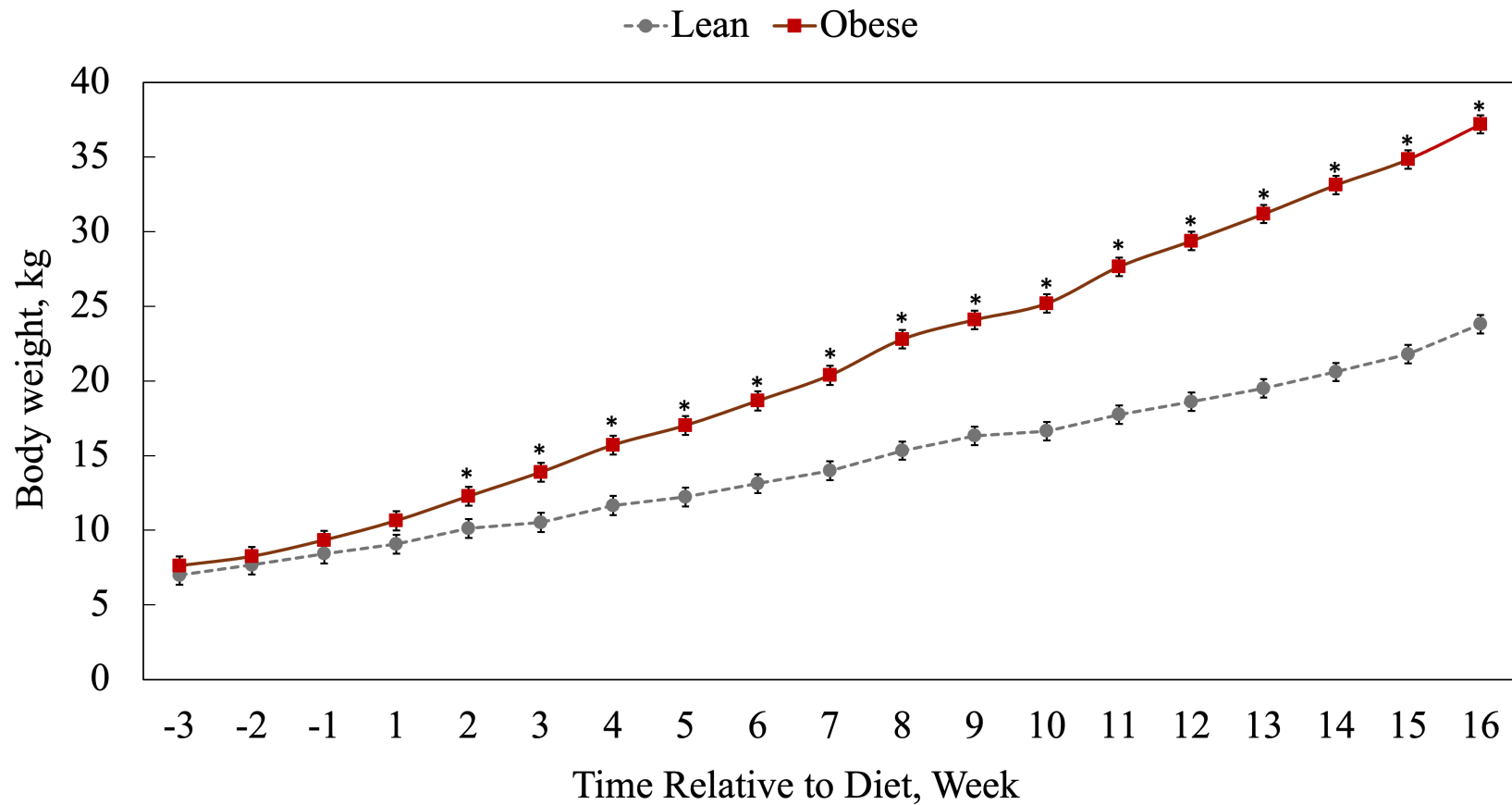


Figure 2.3 Body weight percentage change of Ossabaw pigs (n=32, gilts and boars) from week 0 to week 6 of obese pigs on a weight loss program (OBWL) and lean (LN) pigs (no diet change), supplemented with either isocaloric solution (ISO) or fermented sorghum kombucha (SK) liquid supplementation (TRT*Time, $P \leq 0.001$). Error bars represent standard error mean (SEM). *LS-Means differed for lean (LN-ISO and LN-SK) vs. obese (OBWL-ISO and OBWL-SK) pigs (Tukey-Kramer adjustment).

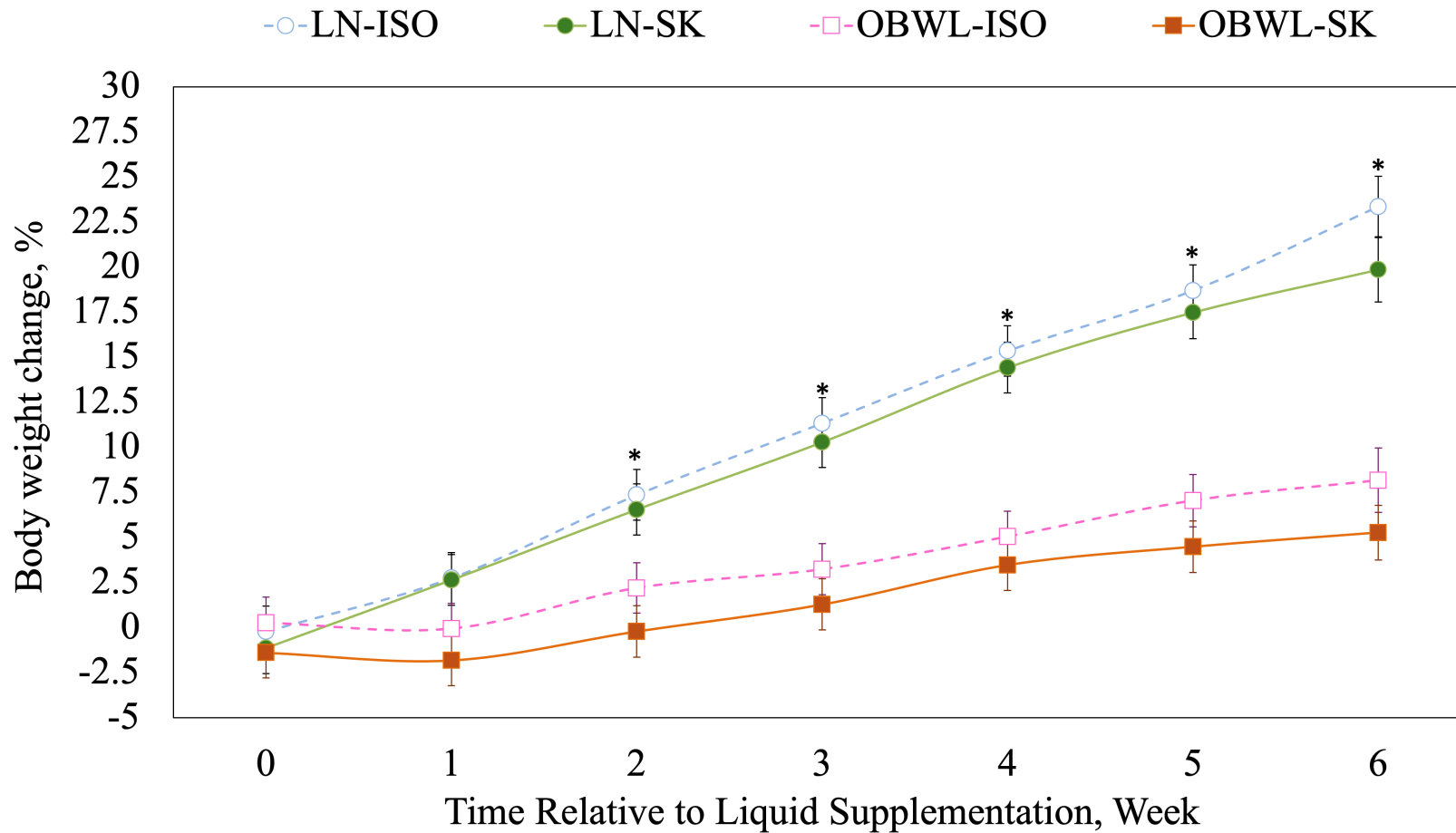


Figure 2.4 A. Average daily gain (kg) and B. Body weight change (%) of Ossabaw pigs (n=32, gilts and boars) of obese pigs on a weight loss program (OBWL) and lean pigs (LN), supplemented with either isocaloric solution (ISO) or fermented sorghum kombucha (SK) liquid supplementation. Error bars represent standard error mean. ^{ab}LS Mans differ ($P \leq 0.05$; Tukey Kramer adjusted) A. Average daily gain (ADG, g/day) over the 6-week liquid supplementation period (TRT, $P \leq 0.001$). B. Percent change in body weight (%) over the liquid supplementation period (TRT, $P \leq 0.001$; Linear, $P = 0.001$, dotted line). Percentage change was calculated using each subject's maximum value prior to supplementation.

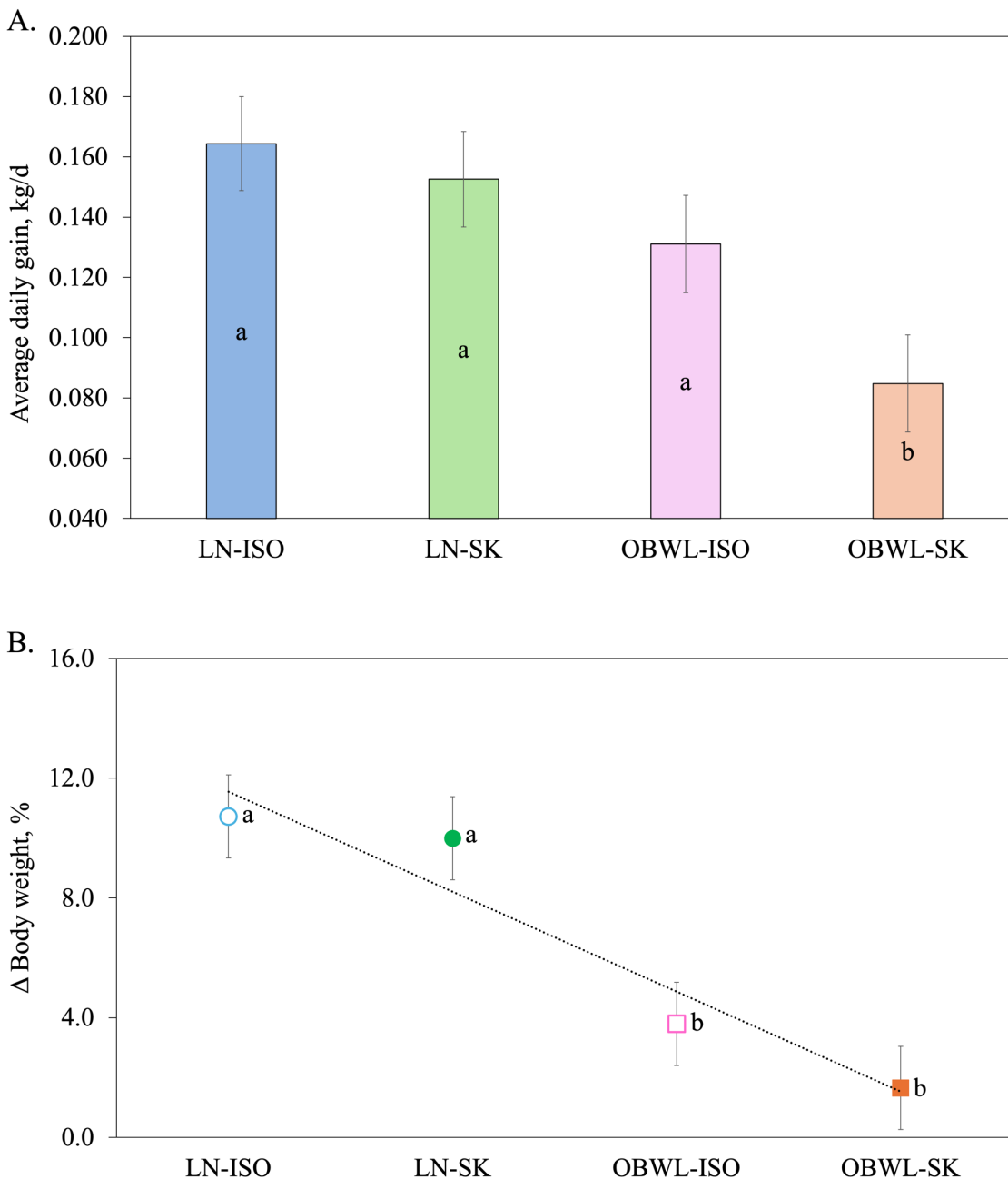


Figure 2.5 A. Total back fat (cm) and B. Back fat percentage change of Ossabaw pigs (n=32, gilts and boars) of obese pigs on a weight loss program (OBWL) and lean pigs (LN), supplemented with either isocaloric solution (ISO) or fermented sorghum kombucha (SK) liquid supplementation. Error bars represent standard error mean. ^{abc}LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted). A. Total back fat (cm) over the 6-week liquid supplementation period (TRT, $P \leq 0.001$). B. Back fat change (%) over the 6-week liquid supplementation period (TRT, $P = 0.003$; TRT*Time, $P \leq 0.001$, Linear $P = 0.002$, dotted line). Percentage change was calculated using each subject's maximum value prior to supplementation.

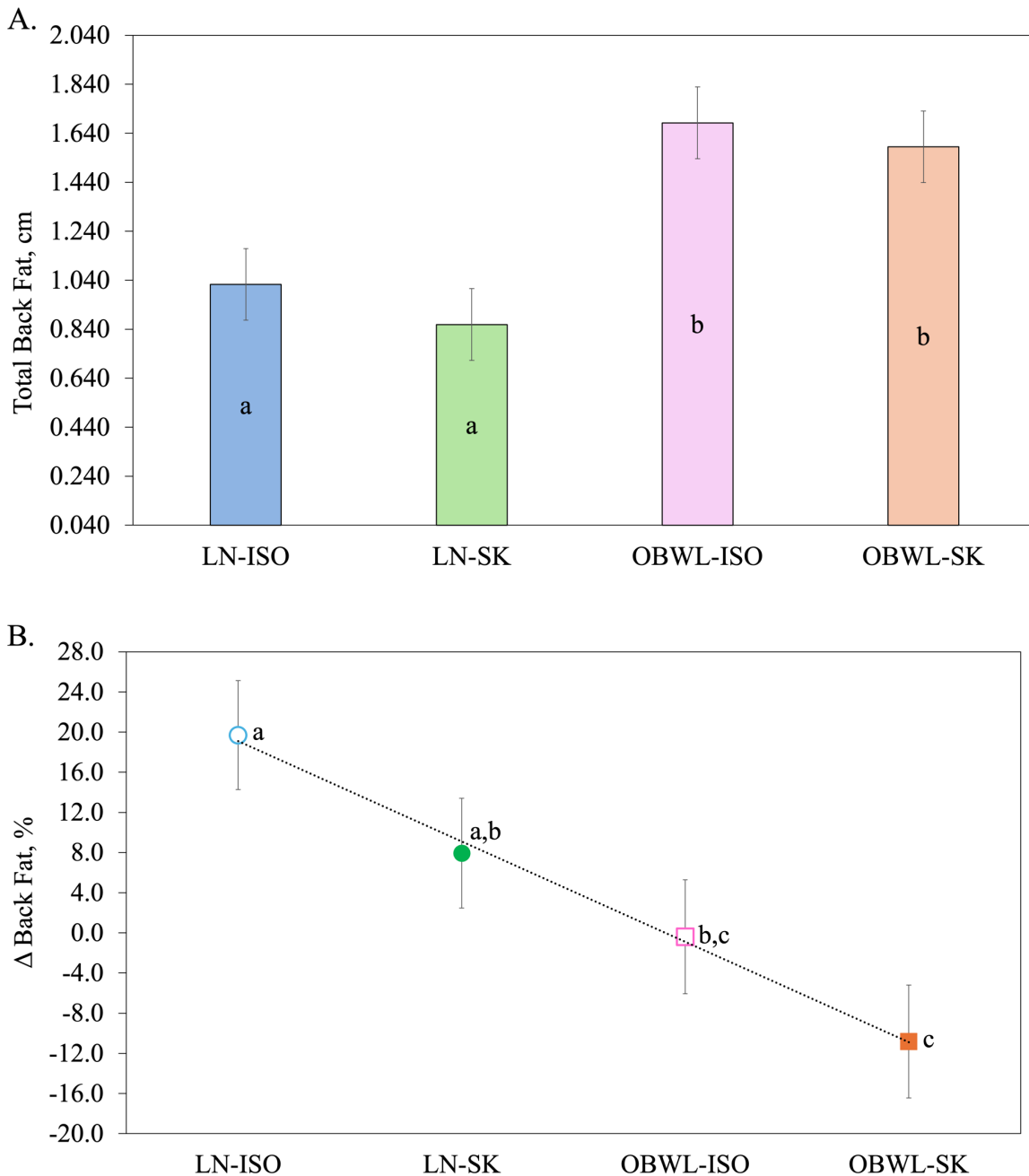
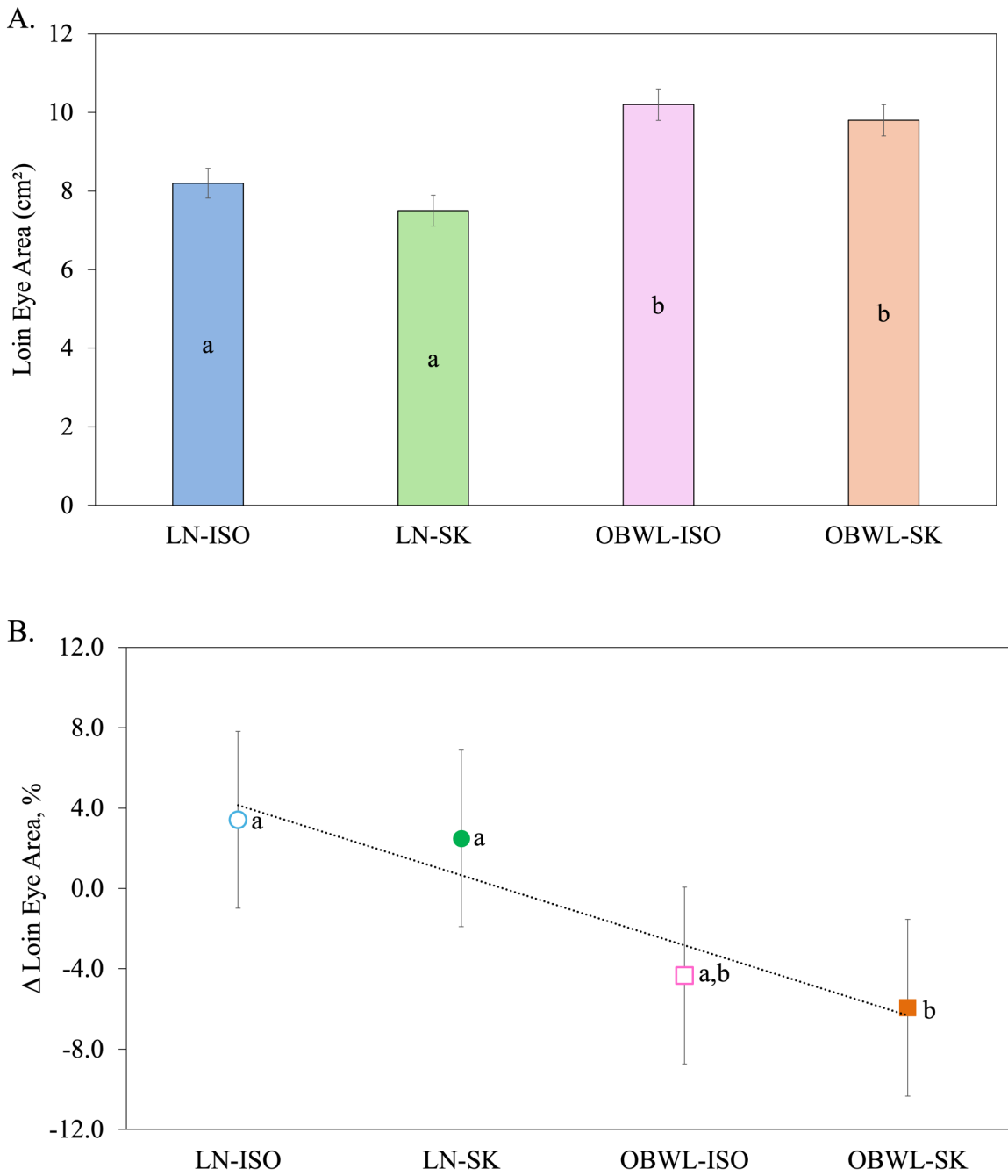


Figure 2.6 A. Total loin eye area (cm²) and B. Loin eye area percentage change of Ossabaw pigs (n=32, gilts and boars) of obese pigs on a weight loss program (OBWL) and lean pigs (LN), supplemented with either isocaloric solution (ISO) or fermented sorghum kombucha (SK) liquid supplementation. Error bars represent standard error mean. ^{ab}LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted). A. Total back fat (cm²) over the 6-week liquid supplementation period (TRT, $P \leq 0.001$). B. Loin eye area change (%) over the 6-week intervention period (TRT, $P = 0.132$; Linear, $P = 0.026$). Percentage change in loin eye area relative to the maximum value recorded before the liquid supplementation period.



Chapter 3 - Sorghum Kombucha Modulates Social and Feeding Behaviors of Lean Pigs and Obese Pigs During Early Weight Loss

Abstract

The objective of this study was to assess whether fermented sorghum kombucha influences social, cognitive, and cooperation behaviors in lean (LN) and obese Ossabaw pigs (Corvus Biomedical, IN; $n = 32$; gilts and boars) on a weight loss program (OBWL), using a cognitive feeder test (CogShape, Master Inc., Ogden, IL). At approximately 3.3 months of age (± 0.52 SD), pigs were placed on one of the four experimental treatments (LN-ISO, LN-SK, OBWL-ISO, or OBWL-SK). Initially, they were fed either a lean (LN) diet (5L80, Purina) or a high-fat Western diet (KT324, Corvus) for 16 weeks. After the dietary induction phase, pigs that received the Western diet were switched to a restricted feeding program (OBWL), matching the same diet as the lean pigs. Both the lean (LN) and calorie-restricted (OBWL) groups were then supplemented for 6 weeks with either an isocaloric control beverage (ISO) or fermented sorghum kombucha (SK; USDA-ARS, Manhattan, KS). Behaviors were recorded through both live observation methods (collected twice daily throughout the experiment) and retrospectively sampled video data (Four 20-minute feedings on weeks -2, 1, 4, and 6 related to liquid supplementation; Observer XT 11.3 software, Noldus Information Technology, Leesburg, VA). At week 4, OBWL-ISO pigs spent a greater proportion of time drinking the isocaloric solution (ISO/SK) compared to other groups (TRT*Time $P = 0.005$). OBWL-SK pigs demonstrated the lowest frequency of switching feed bowls by week 6 (TRT*Time $P = 0.002$). OBWL-SK pigs also exhibited a lower frequency (TRT*Time $P = 0.014$) of drinking liquid supplements in weeks 2 and 4 and had the shortest interval between eating and drinking at week 6 (TRT*Time $P = 0.002$). OBWL-SK pigs took less time to press the correct paddle than LN-SK pigs at week 4

(TRT*Time $P = 0.016$). LN-SK pigs showed the highest frequency of agonistic interactions at week 4 and were more active than OBWL-SK pigs at week 6 (TRT*Time $P < 0.001$). They also spent more time engaging in these agonistic behaviors at week 6 ($P < 0.001$). Latency-to-retreat was highest in OBWL-ISO pigs at week 4 (TRT*Time $P = 0.002$). OBWL-SK pigs consistently showed the lowest pen dominance index percentage, especially at week 2 (TRT*Time $P = 0.001$). On the other hand, LN-SK pigs had the highest individual dominance index percentage at week 1 compared to all obese pigs (TRT*Time $P = 0.048$). Fermented sorghum kombucha may serve as a behavioral modulator in socially housed pigs, particularly by enhancing cognitive engagement and promoting more balanced social dynamics in obese individuals undergoing weight loss. Further research is warranted to determine whether prolonged kombucha supplementation can support behavioral resilience and cognitive flexibility in metabolically compromised subjects, and to elucidate the mechanisms underlying these effects.

Keywords: Sorghum Kombucha; Ossabaw Pig; Obesity; Dominance; Cognition; Feeder

Introduction

Obesity is a complicated physiological and behavioral disorder that has major consequences for animal and human health. Although usually defined in terms of caloric excess and metabolic disturbance, obesity is increasingly understood as a disorder rooted in behavior, especially in how individuals view, desire, and react to food and related rewards (Ramona Guerrieri et al., 2008; Berthoud, 2011; Volkow et al., 2011). Growing use of large animal models, especially pigs, has allowed researchers to investigate these behavioral processes with great translational value. Pigs have strong feeding patterns, learning ability, and social behavior. They also share anatomical, metabolic, and cognitive traits with humans that are quite important

to obesity studies (Lind et al., 2007; Elise Titia Gieling et al., 2011; Elise T. Gieling et al., 2011; Meurens et al., 2012; Roura et al., 2016).

Obese and lean individuals may have quite different behavioral patterns around cognitive flexibility, reward preference, and food drive (Stice et al., 2008; Volkow et al., 2013; Farruggia and Small, 2019; Song et al., 2022). Adult subjects with greater body fat percentages (> 32% in females; > 25% in males), for instance, may show changed sensitivity to delay, less persistence when food access is cognitively difficult, or less curiosity in non-food stimuli (De Jonge et al., 2008; Spyridaki et al., 2016). Factors under growing investigation in both biomedical and agrimedical research, such as systemic inflammation, metabolic inflexibility, or variations in reward processing, can all be connected to these changes (Volkow and Baler, 2015; Guillemot-Legris and Muccioli, 2017; Smith et al., 2018). Furthermore, socially housed animals like pigs interact in sophisticated ways that affect resource availability (Tuchscherer et al., 1998; D'Eath, 2005). Pigs create social hierarchies even in dyads that might affect feeding chances and behavioral patterns (Meese and Ewbank, 1973; Hsia and Wood-Gush, 1984).

When individuals aim to reduce caloric intake during a weight loss process, especially the early stage of weight loss, sugar-sweetened beverages are often among the first targets for replacement (Malik et al., 2013). While diet sodas may offer a calorie-free alternative, they frequently contain high levels of caffeine and synthetic sweeteners with limited nutritional value (Gardner et al., 2012). In contrast, kombucha, a fermented tea beverage, provides modest calories while delivering micronutrients, polyphenols, and live microbial cultures (Greenwalt et al., 2000). Compounds rich in polyphenols are recognized for their impact on metabolic health as well as cognitive function and behavior (Joseph et al., 2005; Gomez-Pinilla and Nguyen, 2012; Lamport and Williams, 2020). Sorghum, particularly pigmented variants, possesses elevated

levels of flavonoids and phenolic acids, which have exhibited antioxidant and anti-inflammatory properties in both animal and human research (Joseph et al., 2005; Kiptanui et al., 2022). Fermentation of sorghum preserves its beneficial components while producing additional probiotic and microbial metabolites that may improve gut-brain axis signaling, a pathway involved in mood regulation, cognitive flexibility, and reward processing (Olivier et al., 2023; Khablenko et al., 2025). Sorghum-based kombucha, still unexplored, is a potential functional beverage for the market, introducing not only a health-oriented option but also expanding the utility of sorghum as a sustainable crop. Given the links between obesity, chronic low-grade inflammation, and altered reward sensitivity, such a beverage may offer a nutritional strategy to support improved, not only body composition, but behavioral regulation in obese subjects (Rezaee et al., 2021; Nelson et al., 2023).

The objective of this study was to evaluate the influence of the sorghum fermented kombucha on social behaviors, cognition, and cooperation in lean and obese pigs on a weight loss program during a cognitive feeder test. The hypothesis was that the sorghum kombucha would impact behaviors among both lean and obese pigs.

Materials and Methods

Animals, Housing, and Husbandry

A total of 32 Ossabaw pigs (16 gilts, 16 boars; Corvus Biomedical, LLC, Crawfordsville, IN) were enrolled using a two-block design (16 pigs per block), with trials occurring from April to October 2023 and January to July 2024, respectively. During the initial phase of the study, pigs were housed at Corvus Biomedical in Crawfordsville, IN. At approximately 6 ± 0.52 months of age, they were transported (8 hours duration) to the Large Animal Research Center (LARC) at Kansas State University (Manhattan, KS) to begin structured data collection. At

LARC, animals were placed in climate-controlled rooms containing 3.7×3.7 m pens (Figure 3.1), each equipped with an automated feeding station (1.6×1.2 m; Figure 3.2). Pen (8 total for each block) assignments were made to ensure an even distribution of treatments and sexes across both sides of the housing room, with four pens per side (Appendix B). Feed was offered twice daily, while water was provided ad libitum. To promote environmental stimulation, pigs had access to a combination of dynamic enrichment items, such as Astros and Lunas (EasyFix, SD, USA), fixed bells, milk jugs, and static enrichment, such as chains and a scratch-mat pole (Figure 3.1).

All procedures were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee (IACUC #4831).

Treatment Assignments

The sample size calculation was based on methods previously described (Martin et al., 1987) and behavioral variance data (Gautier et al., 2020) of male and female minipigs that were: 1) Lean but fed ad libitum a hypercaloric standard diet or high-fat sucrose diet; 2) Obese and fed a western diet before placement on a weight loss intervention strategy (i.e. restricted feed or surgery). Desired differences of behaviors during a memory test (hole board) were 5, 25, 50, and 50% for non-nutritive oral behaviors, test completion, working memory, and test completion time, respectively. Desired differences for preference of sucrose vs. normal chow were set to 10%. The alpha (α) was set to 0.05, and power (1-beta) was set to 0.95 for all measures, which resulted in up to 4 animals per treatment for 4 treatments. For balanced sex (boar or gilt), 8 animals per treatment were used.

At Corvus Biomedical, pigs were at first group-housed and later stratified into treatment pairs at approximately 3.3 ± 0.52 SD months of age. Before diet treatments began, all pigs were fed a standard grower diet (DuMOR Hog Grower, Purina). Prior to assigning dietary treatments, pigs were stratified by sex and body weight to minimize competition within pairs. Individuals in the upper and lower quartiles for body weight were first paired with partners of similar weight and the same sex, while those with intermediate weights were paired randomly using the “RAND” function in Microsoft Excel (Microsoft Corp., Redmond, WA). Each pair was then randomly allocated to a body type category, lean (LN) or obese (OBWL), and subsequently assigned to one of two liquid supplementation treatments for the final six weeks of the study, isocaloric control (ISO) or sorghum kombucha (SK). The allocation was performed by generating random numbers for each pair, sorting the list, and assigning treatments in order to ensure even distribution.

Lean pigs were provided with a calorie-appropriate chow for the entirety of the trial (LabDiet Mini-Pig HF Grower 5L80; Purina Test Diet, Richmond, IN). In contrast, obese pigs were transitioned to a hypercaloric, high-fat Western-style diet (MetS; KT324; Corvus Biomedical, IN) for a 16-week period. Following this phase, they were switched to the same diet and caloric level as the lean group for the final six weeks of the study (Figure 3.3). The standard diet (LabDiet) provided 1,650 kcal per day and was formulated to promote normal growth, containing 9.9% fat (ether extract), 70.3% carbohydrates, and 19.7% protein. By comparison, the Western diet supplied up to 3,400 kcal per day and was specifically designed to promote obesity. In the Western diet formulation, 40.8% of carbohydrate calories were derived from high-fructose corn syrup, fats (classified as ether extract) accounted for 42.9% of the total dietary energy, and protein comprised 16.9%, according to chemical composition analysis (Appendix A).

To minimize gastrointestinal disturbances during diet transitions, a gradual step-up and step-down approach was used, involving 25% dietary changes every three days (progressing through 25%, 50%, and 75% for each transition). Lean pigs were transitioned over a 9-day period from their initial standard grower feed (DuMOR Hog Grower; Purina, Richmond, IN) to the LabDiet formulation, which they consumed for the remainder of the study. After enrollment, obese pigs were switched to a Western-style diet (MetS; KT324; Corvus Biomedical, IN) and remained on it for 16 weeks. After 16 weeks, obese pigs transitioned to the lean diet prior to the liquid supplementation stage (Figure 3.3).

Liquid supplementation occurred during the final six weeks of the trial (Figure 3.3). Both lean pigs and obese pigs in the weight loss phase received two daily doses (500 mL immediately following each meal) of either an isocaloric sucrose solution (ISO; 0.03 kcal/mL) or fermented sorghum kombucha (SK; 0.03 kcal/mL following microbial nutrient processing). Supplements were offered 3–5 minutes after feed delivery in bowls positioned next to the feed bowls. Both formulations had been previously developed and validated (Nelson et al., 2023) and were supplied by the United States Department of Agriculture – Agricultural Research Service (USDA-ARS, Manhattan, KS).

Daily and Weekly Routines

Pigs were maintained on a standardized weekly schedule to facilitate data collection and reduce stress associated with handling. Feeding sessions took place daily at 0800 and 1600 h, followed by the removal of fecal matter and the cleaning of feeders. On Monday through Wednesday mornings, before feeding, each pig completed an individual trial in an 8-arm radial maze as part of a larger research initiative (Scott, 2025). Body weights were recorded on Mondays prior to maze testing. Each Thursday, pigs were weighed before being placed in a

containment sling (Panepinto, Loveland, CO), a humane swine restraint system originally developed in the 1960s, to enable consistent positioning for ultrasound imaging while minimizing physical pressure and stress. Every 15 days, blood samples were also collected on Thursday mornings, at the same time as the ultrasound image collection, for subsequent analysis. Between feeding periods on Thursdays, pairs were temporarily moved to holding pens while their home pens and feeders were sanitized. From Friday to Sunday, only the core tasks of feeding and liquid supplementation were carried out in the morning and evening.

CogShape Feeding System

A 4 bowl in-pen cognition feeder, CogShape (Figure 3.2; CogShape, Hulbert, Manhattan, KS and ShapeMaster Inc., Ogden, IL) was further developed after the first prototype was tested (Nelson, 2024). This feeder, formally disclosed to the Kansas State University Innovation Center, was designed to assess decision-making and behavioral flexibility in Ossabaw pigs under different diet and fluid treatments. A four paddle, four bowl CogShape feeder was installed directly within each pen and enclosed by a solid-sided stall to reduce distractions and maintain symmetry across test environments (Figure 3.1). The feeder measured 165.1 cm in length by 104.06 cm in width, with four front-positioned bowls, two on each side, each lateral section measuring 43.18 cm in width (Figure 3.2). In Block 2024, the CogShape area was installed over slatted green flooring (RipGrid commercial-grade, open-slot plastic flooring), which enhanced cleanliness, added non-cement resting, and minimized debris accumulation.

The CogShape system consisted of four paddles, each connected to an internal mechanism that released feed rewards into bowls. Paddles were programmed as active according to each phase of the feeder cognitive test (Pair or Individual; Acclimation, Condition, Acquisition, and Reversal; Figure 3.4), during each session, requiring pigs to press the correct

paddles to receive feed into a connected feed bowl. Incorrect paddles produced no immediate reward. To signal a successful response, indicator lights were positioned between each pair of hoppers on the left and right sides of the feeder, illuminating and producing a brief sound cue when the correct paddle was activated. This visual feedback helped technicians confirm paddle accuracy in real time and during video review. The feeder system was connected to a central programmable logic controller (PLC), which enabled automated control of the paddle-response patterns and precise timing, and quantity of feed released. The PLC was programmed prior to each session to define which paddles would be active and to control reward delivery logic, ensuring consistency and adaptability across sessions and treatments.

Feed was manually distributed into the correct feed bins prior to each session. The amount of feed per test ranged from 225 to 634 grams per correct hopper, depending on each pair's dietary phase. During tests, food was only released upon pressing the correct paddle. During the liquid supplementation treatment phase, one of the bowls on each side was assigned as a fluid reward station (isocaloric solution or sorghum kombucha). These bowls were not linked to paddle activation. Instead, it was funneled into the designated bowl approximately 3 to 5 minutes after the feed dispensed. In the 2024 block, some automated feeders experienced mechanical issues in which feed was not fully dispensed during active paddle presses. To maintain consistency in reward delivery, a split-feed protocol was adopted. Half of the feed was manually placed into the hopper, and the remaining portion was funneled into the same feed bowl approximately 2–3 minutes later. This timing mirrored that used for the delayed liquid supplementation reward. Importantly, these feed-based funneling events occurred only during the feeding sessions that took place before the liquid supplementation period. As a result, feed and liquid supplementation funneling never occurred at the same section. All feeder components and

pen surfaces were thoroughly cleaned between sessions to eliminate olfactory cues and prevent cross-contamination.

Animal Handling and Testing Procedure

The feed sessions took place twice daily, at 0800 and 1600 h. At the start of each feed session, one trained technician remained at the observation computer for live data collection, and two trained technicians entered the pen. Within the pen, one technician used a hog board to gently contain and manage the pigs' movement, while the second technician removed the two front guillotine panels of the feeder to expose the paddles and bowls. Once the feeder was open, the pigs were calmly released and allowed to interact with the CogShape device. Both technicians exited the pen after the session began to avoid interference with pig behavior. In the majority of the cases, pigs engaged independently with the feeder system. However, if no interaction occurred within 3 minutes, shaping procedures were employed to re-engage the pigs. Shaping involved visual cues such as pointing toward the paddles or using a sticker to draw attention to the correct paddle. Shaping was only used when necessary and was applied consistently across sessions to avoid introducing bias.

To ensure consistent and coordinated operations, all 3 technicians, the 2 inside the pen and the technician stationed at the observation computer, used walkie-talkies throughout the sessions. This communication system allowed real-time coordination for actions such as initiating shaping of the designed paddle, funneling, and signaling when cleaning should begin. This protocol helped reduce human error and maintain consistency across trials. Each session was recorded using a customized network video recorder (NVR) that captured footage continuously at 30 frames per second with a resolution of 1080 pixels. A single bullet camera (GeoVision Model GV-EBL4702-2F; GeoVision, Irvine, CA) was mounted at a 90-degree angle

toward the feeder area, enabling a full view of pig interactions with the CogShape system and bowl areas. This footage was used to verify real-time observations and conduct detailed post-session behavioral analysis. The order of the feeder sessions remained consistent throughout the study, beginning with Pen 8 and proceeding in reverse numerical order to Pen 1 (Appendix B). This predictable routine helped reduce anticipatory stress and allowed for consistent environmental and behavioral conditions across sessions. The minimal-handling protocol was designed to prioritize pig welfare and test reliability by reducing stress and ensuring that pigs could perform the task under low-pressure, familiar conditions.

CogShape Timeline

The CogShape cognitive feeding test was implemented with distinct phases for acclimation, conditioning, acquisition, reversal, and alternating between paired and individual feeding testing formats. All weeks are reported relative to the start of liquid supplementation (week 1), with negative values (e.g., week -10) referring to the weeks prior to supplementation and positive values indicating weeks following supplementation. CogShape testing started 10 weeks prior to the liquid supplementation, during the period that the subjects were housed at the LARC facility. Paddles were consistently labeled A to D from the pig's left to right inside the feeder (Figure 3.2). During the -10 week (time relative to liquid supplementation), pigs were introduced to the CogShape feeder to familiarize them with the feeder's physical structure and bowl locations (Acclimation; Figure 3.4). Although the feeder was present and functional, feed was pre-placed into all four bowls prior to each session, allowing pigs to associate the feeder area with food availability without requiring a lever press. No lights, sounds, or paddle-based interactions were active during this week. From weeks -9 through -6 (time relative to liquid supplementation), all four paddles (A–D) were activated and capable of dispensing feed

(Condition; Figure 3.4). Pigs were tested in pairs, and each session began with a 3-minute exploratory period. During this time, pigs were given the opportunity to discover the paddle-pressing mechanism. If no correct paddle interaction occurred after 3 minutes, trained technicians employed shaping procedures (e.g., physically or with a sticker - pointing or guiding) the pig toward the correct paddle or activating the paddle manually to demonstrate reinforcement.

Beginning in week -5, pigs entered the acquisition phase (Figure 3.3), where only two paddles were programmed as correct (active) per session. The correct paddles dispensed feed upon activation and were accompanied by a light and sound cue. Pressing an incorrect paddle resulted in no stimuli and no feed delivery. At the same time that each pig pair (pen) was assigned to one of the 4 treatments, they were also randomly assigned one of several acquisition task formats (Figure 3.4). The paddle configurations used during this phase were: Labeled as paddles A, B, C, and D from the pig's left to right, the spatial layout of patterns was also described using combinations (Figure 3.4). From weeks -4 to -2, pigs continued the acquisition task but were tested individually. The two-correct-paddle format remained, and only one pig per pen entered the feeder area at a time. The order of testing alternated daily within each pen, ensuring balanced exposure. In weeks -1 to 1, pigs returned to pair testing while continuing the acquisition phase. The same two-correct-paddle configurations and stimulus rules were applied.

The reversal phase was added to increase difficulty after latency outcomes reached floors (i.e. latency-to-paddle < 1 second). In the reversal phase, at week 2, each pen's prior correct paddle combinations were inverted, requiring pigs to unlearn the previous correct choices and identify the new correct paddles (Figure 3.4). At week 2, pigs had 3 days of individual sessions, then went back to pair sessions. As in the prior individual phase, the order of pigs was alternated

daily to control for order effects. From week 3 until the conclusion of the experiment (week 6 – time relative to tea), pigs remained in pair testing within the reversal paradigm. Paddle combinations continued to follow the reversed patterns, and reinforcement protocols remained the same. CogShape design and timeline allowed assessment of learning, memory, and cognitive flexibility under varying treatment conditions.

While individual training and testing sessions were incorporated to support learning and assess behavior under controlled conditions, only data collected during pair testing were analyzed in this study. Most feeder sessions lasted approximately 20 minutes. After the conditioning phase, shaping interventions were infrequent and used only when necessary. All shaping events were noted by the observer, and their occurrence declined as pigs became familiar with the task.

Live Observation

During each feeding session, observers recorded real-time responses for each pig, classifying them into one of three categories based on their task performance: 1) Pressed both correct paddles, indicating that the pigs did all the correct test work. 2) Pressed one correct paddle, indicating the pig did one correct work or equal correct work between the pig pair. 3) Did not press a paddle, indicating the pig did not do any correct work. Each pig was assigned to one of these three categories per observation session. The live observational data were used to provide a rapid, binary outcome-based summary of feeder engagement and performance. Only live observation data from paired feeding sessions were included in the analysis. The categorical outcome data were exclusively used for Chi-square test (Chi-Square Test Calculator, 2025) analyses to evaluate differences in cognitive task engagement across treatment groups,

particularly enabling the assessment of contrafreeloading tendencies. The Chi-square analyses are described in detail in the following sections.

Video Behavioral Quantification

Video data were sampled from pair testing (4 meals per week, Saturdays and Sundays) on weeks -2, 1, 2, 4, and 6 relative to liquid supplementation (Figure 3.3) and subsequently coded using Observer XT 11.3 software (Noldus Information Technology, Leesburg, VA). Each video was approximately 20 minutes in length and captured during feeding sessions conducted as part of the CogShape cognitive feeding test. A pre-established ethogram was used to classify behavioral events and states, including cognitive task behaviors (e.g., correct/incorrect paddle presses), feeding and drinking preferences (relative to liquid supplementation, not water), social interactions, non-nutritive oral behaviors (NNOB), jumping, light cues from the feeder system, and human actions such as funneling liquid supplementation or feed.

Each behavior was classified as either states (duration) or point events (frequency per session), and behaviors were coded independently for each pig within the pen. These behaviors were combined to form a mutually exclusive coding scheme, ensuring that only one behavior was assigned to each pig at any given moment in the video timeline. A primary and secondary ethogram, along with the corresponding coding keys, are provided in Table 3.1 and Table 3.2, respectively. Both the primary and secondary ethograms were incorporated into a single coding scheme within Observer XT software to ensure synchronized behavioral tracking. The primary ethogram focused on the behaviors of the pig subjects (Table 3.1), while the secondary ethogram captured the actions of the human handler when funneling (Table 3.2) and the feeder system (lights). Behaviors were defined and annotated beginning immediately after the pig entered the feeder area and their head crossed the threshold (Figure 3.5).

Three trained ethologists participated in video scoring. Prior to full dataset coding, a reliability test was conducted using a representative sample of six videos, covering twelve pigs from both treatment groups. Observers were blinded to treatment status to reduce bias. Key behavioral metrics evaluated included the total duration of each behavior (how long it occurred), frequency (how often it occurred), and latency (the time it took for the behavior to first appear). To assess inter-observer agreement, Intraclass Correlation Coefficients (ICCs) were calculated in R Studio (Version 4.3.2; Posit Software, PBC, 2024). A two-way model for consistency based on Czycholl et al., 2019 was applied, which evaluates the degree to which different raters give consistent scores, assuming each subject (video) is rated by the same set of observers. ICC values range from 0 to 1, with higher values indicating stronger agreement. In this study, the mean ICC for total duration was 0.918, indicating near-perfect agreement between observers in coding behavior length. The mean ICC for latency was similarly high at 0.968, reflecting strong consistency in identifying the onset of behaviors. The mean ICC for frequency was slightly lower at 0.845 but still within the range considered good reliability for behavioral research. Overall, these values confirm excellent inter-observer reliability, particularly for the duration and timing of behaviors.

Once reliability was established, the 320 video sessions were randomized by pen and divided among the three observers. Since each session involved two pigs, a total of 640 individual behavioral recordings were analyzed. All videos were drawn from paired feeding sessions conducted on weekends, including two weekends prior to the introduction of tea treatment and several weekends thereafter. Specifically, videos from weeks -2, 1, 2, 4, and 6 (relative to the start of liquid supplementation), allowing for comparative behavioral analysis across time. As described previously, at week -2 and week 1, pigs were engaged in the

acquisition phase of the CogShape task, while weeks 2, 4, and 6 corresponded to the reversal phase. To maintain consistency across observers and sessions, a standardized coding order was strictly followed during each video review. Observers began by coding Pig 1, followed by Pig 2 from the same session, both were identified by ear tag color in Observer XT. Once both pigs were coded, observers proceeded to record feeder light events, such as the activation and deactivation of the left or right feeder-side indicators. Lastly, all human actions, including funneling of feed or tea into specific bowls, were annotated. This structured workflow ensured a uniform approach to behavioral coding, minimized variability between observers, and improved the accuracy of time-stamped behavioral events. All video sessions were coded in half-speed playback (15 frames per second) to enhance precision in identifying subtle or rapid behaviors.

To ensure standardized observation length, the end time of each coded session was pre-established based on experimental conditions. In week -2, when no liquid supplementation was administered, end time criteria differed slightly by block. In Block 1 (2023), the coding ended 5 minutes after both feeder-side lights were turned off. In Block 2 (2024), due to the implementation of a split-feed protocol, coding ended 5 minutes after the feed was funneled into both bowls. Importantly, the 5-minute interval began once the funnel was no longer visible within the feeder area. For weeks 1, 2, 4, and 6, during which liquid supplementation funneling occurred, the end time was consistently set to 5 minutes after the liquid supplementation funneling event in both drink bowls. Similarly, the 5-minute interval began after liquid supplementation funneling had concluded, and the funnel was no longer visible within the feeder area, ensuring consistency in behavioral observation across all sessions. The total duration of each video observation session, once coded, was approximately 10 to 15 minutes.

Dominance Index Calculation

The same videos used for behavioral quantification, coded in Observer XT (640 individual behavioral recordings), were also used to calculate the dominance index. Dominance status was determined for each pig pair using a modified dominance index based on the method described by Bateson and Martin (2021) and Zumpe and Michael (1986). The method was adapted to reflect dyadic interactions occurring exclusively between two animals housed together, with a focus on the frequency of social behaviors observed during paired feeding sessions, similar method described by (Nelson, 2024). To calculate the dominance index, a matrix was first constructed for each pen using the observed frequencies of advances (approach behaviors) and displacements (instances where one pig moved away in response to the other's advance) exhibited by each pig. Frequencies were aggregated across all observed sessions for each pen, producing a total number of advances and displacements per pen. For each individual pig, the number of advances was divided by the total number of advances within the pen and multiplied by 100 to yield a percent advance. The same calculation was performed using displacement behaviors to yield a percent displacement. These values were then averaged by summing the two percentages and dividing by two (number of pigs within the pen), resulting in a final dominance index for each pig. The stepwise equations used to calculate the dominance index are presented below.

- 1) Percent Advance. For a dyad (i, j), where both pigs have advanced:

$$Adv_{ij} = \left(\frac{A_{ij}}{A_{ij} + A_{ji}} \right) \times 100$$
$$Adv_{ji} = \left(\frac{A_{ji}}{A_{ij} + A_{ji}} \right) \times 100$$

where A_{ij} is the number of times pig i advanced toward pig j . If $A_{ij} + A_{ji} = 0$, the advance metric is excluded for the dyad.

- 2) Percent Displacement. For the same dyad (i, j), where both pigs have displaced each other:

$$Dis_{ij} = \left(\frac{D_{ij}}{D_{ij} + D_{ji}} \right) \times 100$$

$$Dis_{ji} = \left(\frac{D_{ji}}{D_{ij} + D_{ji}} \right) \times 100$$

where D_{ij} is the number of times pig i displaced pig j . If $D_{ij} + D_{ji} = 0$, the displacement metric is excluded for the dyad.

- 3) Within-Pair Dominance Index. For dyad (i, j):

$$P_{ij} = \begin{cases} \frac{(Adv_{ij} + Dis_{ij})}{2}, & \text{If both advance and displacement exist} \\ Adv_{ij}, & \text{if only advance exists} \\ Dis_{ij}, & \text{if only displacement exists} \end{cases}$$

The pig with the higher dominance index within each pen was labeled dominant, and the one with the lower index was labeled submissive. The use of percentages allows for a scaled measure of dominance, enabling comparisons across dyads while still preserving a clear binary classification.

Statistical Analysis

Statistical analyses for behavioral outcomes from Observer XT were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC). Prior to modeling, data distributions were assessed for normality using the Shapiro-Wilk test within the UNIVARIATE procedure. Repeated measures analyses were conducted using the MIXED procedure with residual maximum likelihood (REML) as the estimation method. Degrees of freedom were adjusted using

the Satterthwaite approximation to account for variability at the pen level. Several covariance structures were tested for repeated measures modeling, including compound symmetry (CS), first-order autoregressive [AR(1)], heterogeneous autoregressive [ARH(1)], and ante-dependence [ANTE(1)]. The optimal structure was selected based on the lowest Akaike Information Criterion (AIC) value. The experimental unit was the pen, composed of a pair of pigs matched by sex and weight and randomly assigned to one of four treatments. Pen was included as the subject effect, and repeated measures were defined by week or session nested within week, depending on the behavioral variable analyzed. Fixed effects in the model included treatment, block, week, and their interactions. Sex (boar or gilt) was initially considered as a covariate and retained only if the associated P -value was ≤ 0.10 . Pen nested within treatment was included as a random effect to account for variability not explained by fixed factors. Model-generated least squares means (LSMeans) $\pm$ standard error of the mean (SEM) were compared using the Tukey-Kramer adjustment to control for family-wise Type I error. Statistical significance was set at $P < 0.05$. Trends were noted for P -values between 0.05 and 0.10 if supported by biological interpretation.

Live categorical outcomes, including jump behavior (frequency), were evaluated using a Chi-square test via an online calculator (Chi-Square Test Calculator, 2025), applying a significance threshold of $P < 0.05$.

Results

Feeding, Drinking, and Jumping Behavior

The eat duration reflected the total time pigs engaged in feed consumption during the observation period (10-15 minutes). LN-ISO and OBWL-ISO decreased their eat percentage duration from their week -2 value at weeks 2, 4, and 6 (TRT*Week $P < 0.001$; Table 3.3; Figure 3.6). LN-SK decreased their eat percentage duration from their week -2 value at all the

subsequent weeks (weeks 1, 2, 4, and 6; TRT*Week $P < 0.001$; Table 3.3; Figure 3.6). OBWL-SK only decreased from their week -2 value at week 2 (TRT*Week $P < 0.001$; Table 3.3; Figure 3.6). At week 1, OBWL-ISO had a higher eat percentage duration compared to all LN pigs (TRT*Week $P < 0.001$; Table 3.3; Figure 3.6). During the same week, OBWL-SK also exhibited a higher eat percentage duration than LN-SK (TRT*Week $P < 0.001$; Table 3.3; Figure 3.6). At week 4, OBWL-ISO displayed a lower eat percentage duration compared to both their counterparts (OBWL-SK) and LN-ISO pigs (TRT*Week $P < 0.001$; Table 3.3; Figure 3.6). In contrast, in the same week, OBWL-SK showed a higher eat percentage duration compared to LN-SK. By week 6, OBWL-ISO exhibited the lowest eat percentage duration among all groups (TRT*Week $P < 0.001$; Table 3.3; Figure 3.6).

Feed bowl switching frequency referred to how often pigs alternated between the left and right feed bowls during the observation period (10-15 minutes). LN-SK increased their feed bowl switching frequency from week -2 value at weeks 2, 4, and 6 (TRT*Week, $P = 0.003$; Table 3.4; Figure 3.7). Both LN-ISO and OBWL-ISO increased their feed bowl switching frequency from week -2 value only at week 6 (TRT*Week, $P = 0.003$; Table 3.4; Figure 3.7). At weeks -2 and 1, OBWL-ISO exhibited a higher frequency of feed bowl switching compared to OBWL-SK (TRT*Week, $P = 0.003$; Table 3.4; Figure 3.7). By week 6, OBWL-SK expressed the lowest feed bowl switching frequency (TRT*Week, $P = 0.003$; Table 3.4; Figure 3.7).

The drink duration reflected the total time pigs spent consuming the liquid supplementation (ISO/SK) during the 10-15 minute observation period. OBWL-ISO increased their drink percentage duration compared to their week 1 value at all subsequent weeks (weeks 2, 4, and 6; TRT*Time $P = 0.005$; Table 3.3; Figure 3.8). At week 1, OBWL-ISO had a lower drink percentage duration compared to all LN pigs (TRT*Time $P = 0.005$; Table 3.3; Figure 3.8). By

week 2, OBWL-ISO drink percentage duration was higher than OBWL-SK (TRT*Time $P = 0.005$; Table 3.3; Figure 3.8). At week 4, OBWL-ISO demonstrated the highest drink percentage duration compared to all other groups (TRT*Time $P = 0.005$; Table 3.3; Figure 3.8).

Drink frequency referred to the number of times pigs transitioned from any other behavior into a distinct drinking bout of liquid supplementation (ISO/SK) during the observation period (10-15 minutes). LN-SK increased their drink frequency from week 1 value at all the subsequent weeks (weeks 2, 4, and 6; TRT*Week, $P = 0.014$; Table 3.4; Figure 3.9). LN-ISO increased their drink frequency from week 1, at weeks 2 and 6 (TRT*Week, $P = 0.014$; Table 3.4; Figure 3.9). In contrast, OBWL-ISO decreased their drink frequency from week 1 value only at week 4 (TRT*Week, $P = 0.014$; Table 3.4; Figure 3.9). At week 1, OBWL-ISO showed higher drink frequency compared to all other groups (TRT*Week, $P = 0.014$; Table 3.4; Figure 3.9). At weeks 2 and 4, OBWL-SK presented lower drink frequency compared to all other treatment groups (TRT*Week, $P = 0.014$; Table 3.4; Figure 3.9). At week 6, OBWL-SK continued to have a lower drink frequency compared to both OBWL-ISO and LN-ISO (TRT*Week, $P = 0.014$; Table 3.4; Figure 3.9).

The percentage of observations for latency to drink referred to the proportion of the total observation period that elapsed before the pig initiated its first drinking bout of liquid supplementation. A higher percentage indicated a longer delay before drinking began. In the context of the current study, a shorter latency to drink may be indicative of a preference for the liquid supplementation over the feed. LN-SK tended to increase their latency to drink percentage at week 2 compared to their week 1 value (TRT*Week, $P = 0.059$; Table 3.5; Figure 3.10). Conversely, OBWL-SK tended to decrease their latency to drink percentage from their week 1 value at weeks 4 and 6 (TRT*Week, $P = 0.059$; Table 3.5; Figure 3.10). At week 4, OBWL-SK

tended to have a lower latency to drink percentage compared to LN-ISO (TRT*Week, $P = 0.059$; Table 3.5; Figure 3.10). At week 6, OBWL-SK tended to have the lowest latency to drink percentage among all groups (TRT*Week, $P = 0.059$; Table 3.5; Figure 3.10).

The eat-to-drink interval represented the amount of time, expressed as a percentage of the observation period, that elapsed between the onset of eating and the initiation of drinking the liquid supplementation. In other words, it reflected how long it took pigs to switch from eating to drinking after the liquid supplementation was funneled into the bowl and may also serve as an indicator of the pig's preference for the liquid supplementation relative to feed. Only OBWL-SK showed a decrease in their eating-to-drinking interval from their week 1 value at week 6 ($P = 0.024$; Table 3.5).

A Chi-square analysis was conducted to assess changes in the frequency of jump behavior, also considered an indicator of preference, before and after the introduction of the liquid supplementation. There were 85 instances of the pigs jumping up on the feeder. The frequency of jumping increased in OBWL-SK and LN-SK pigs after applying the liquid supplementation ($P < 0.001$; Table 3.6). Only 1 instance of jump behavior was observed among LN-ISO after the liquid supplementation was applied. Before the solution was applied, there were 17 instances within the LN-ISO group ($P < 0.001$; Table 3.6). Among pigs that display jump, fermented sorghum kombucha increased this reference behavior while the isocaloric solution decreased the behavior ($P < 0.001$; Table 3.7).

Non-nutritive oral behaviors (NNOB) in this context indicated oral activity directed toward non-feed objects (e.g., feeder structure) that may reflect underlying motivational states or redirected foraging behavior. At weeks 2 and 4, OBWL-ISO decreased their NNOB percentage duration from their week -2 value (TRT*Week, $P = 0.019$; Table 3.3). At week 6, only OBWL-

SK increased their NNOB percentage duration from their week -2 value (TRT*Week, $P = 0.019$; Table 3.3).

Cognitive Behavior

Latency to eat (% of observations) referred to the proportion of the total observation period that elapsed before the pig initiated its first feeding bout. A higher percentage indicated a longer delay before feeding began, while a lower percentage reflected earlier engagement with the feed. In the current experiment, initiating the consumption of feed was contingent on one of the pigs correctly pressing the paddle to release feed; latency to eat also served as an indicator of cognitive performance. OBWL-SK tended to have a higher latency to eat percentage compared to all other treatments at week -2, before the liquid supplementation period started, and this pattern persisted at weeks 1 and 2 (TRT*Week, $P = 0.066$; Table 3.5). All treatment groups tended to decrease their latency to eat percentage from their week -2 value at the subsequent weeks (TRT*Week, $P = 0.066$; Table 3.5).

In addition to measuring latency to eat, latency to correct paddle offered a more direct measure of the initial decision-making. The percentage of observations of latency to press the correct paddle represented the proportion of the total observation period that elapsed before the pig successfully pressed the correct paddle to trigger the release of feed, reflecting the pig's ability to identify and interact with the correct paddle to obtain feed, thereby serving as a clearer indicator of learning, memory, and task execution within the cognitive feeder system. A lower latency indicated quicker task engagement and may reflect improved cognitive performance, while a higher latency suggested a delayed response or reduced engagement with the cognitive task. At week -2, OBWL-ISO and OBWL-SK presented a greater latency percentage to push the correct paddle than LN-ISO and LN-SK (TRT*Week, $P = 0.016$; Table 3.5; Figure 3.11).

OBWL-ISO and OBWL-SK decreased their latency percentage to press the correct paddle from their week -2 values at all subsequent weeks (weeks 1, 2, 4, and 6; TRT*Week, $P = 0.016$; Table 3.5; Figure 3.11). At week 4, OBWL-SK and LN-ISO showed the lowest latency percentage to press the correct paddle, while LN-SK displayed the highest latency percentage (TRT*Week, $P = 0.016$; Table 3.5; Figure 3.11).

Agonistic Interactions During Feeding Cognitive Test

Agonistic frequency referred to the number of distinct agonistic interactions (advances and retreats) recorded during the observation period (10-15 minutes). A higher frequency indicated more frequent social conflict or competition. Conversely, a lower frequency suggested fewer social challenges or a more stable social relationship between pen mates. LN-ISO reduced their agonistic frequency from their week -2 value at weeks 1 and 4 (TRT*Week, $P < 0.001$; Table 3.8; Figure 3.12), while OBWL-ISO showed reductions only at week 1 (TRT*Week, $P < 0.001$; Table 3.8; Figure 3.12). At week 4, LN-SK demonstrated the highest agonistic frequency compared to all other groups (TRT*Week, $P < 0.001$; Table 3.8; Figure 3.12), and remained elevated at week 6 relative to OBWL-SK (TRT*Week, $P < 0.0001$; Table 3.8; Figure 3.12).

Agonistic duration percentage of observations represented the proportion of the total observation period during which pigs were actively engaged in agonistic behaviors, such as advancing toward or retreating from the pen mate. A higher percentage suggested extended periods of social tension or competition, while a lower percentage may indicate brief or infrequent conflicts and potentially more stable social dynamics. Reductions in agonistic duration percentage from their week -2 value were evident in OBWL-SK across all subsequent weeks (weeks 1, 2, 4, and 6; TRT*Week, $P < 0.001$; Table 3.8; Figure 3.13). OBWL-ISO showed reductions at weeks 1, 4, and 6 (TRT*Week, $P < 0.001$; Table 3.8; Figure 3.13). LN-SK

showed decreases at weeks 1 and 2, while LN-ISO had lower durations at weeks 4 and 6 (TRT*Week, $P < 0.001$; Table 3.8; Figure 3.13). At week 4, LN-SK had a longer agonistic duration percentage than OBWL-SK (TRT*Week, $P < 0.001$; Table 3.8; Figure 3.13), and by week 6, LN-SK had the highest agonistic duration percentage among all treatments (TRT*Week, $P < 0.001$; Table 3.8; Figure 3.13).

Latency to agonistic (% of observations) referred to the proportion of the total observation period that elapsed before the first agonistic behavior (e.g., advance or retreat) was observed. A higher percentage indicated a longer delay before social conflict began, which may suggest reduced competition, stable social hierarchy, or low motivation to assert dominance. Conversely, a lower percentage reflected a shorter delay, suggesting earlier onset of social tension or dominance-related behavior within the pair. All treatment groups except OBWL-ISO increased their latency to agonistic percentage from their week -2 value at weeks 4 and 6 (TRT*Week, $P = 0.004$; Table 3.9; Figure 3.14). In addition, LN-SK and LN-ISO also showed increases at weeks 1 (TRT*Week, $P = 0.004$; Table 3.9; Figure 3.14). OBWL-ISO only presented an increase in latency to agonistic percentage from their week-2 value at week 6 (TRT*Week, $P = 0.004$; Table 3.9; Figure 3.14). At week 1, LN-SK had a higher latency to agonistic percentage than OBWL-SK (TRT*Week, $P = 0.004$; Table 3.9; Figure 3.14). By week 4, LN-ISO exhibited the highest latency among all groups (TRT*Week, $P = 0.004$; Table 3.9; Figure 3.14).

Latency to retreat (% of observations) represented the proportion of the total observation period that elapsed before the first retreat behavior was observed. The metric provided insight into when a pig first exhibited submissive or avoidant behavior in response to its pen mate. A longer latency (higher percentage) suggested delayed submission, potentially indicating higher

confidence or willingness to maintain position, while a shorter latency (lower percentage) reflected early withdrawal, which may signal lower dominance status or reduced motivation to compete. LN-SK increased their percentages from their week -2 value across all subsequent weeks (weeks 1, 2, 4, and 6; TRT*Week, $P = 0.003$; Table 3.9; Figure 3.15). LN-ISO also showed increased retreat latency at weeks 1, 4, and 6 (TRT*Week, $P = 0.003$; Table 3.9; Figure 3.15). At week 1, OBWL-SK had a lower latency to retreat percentage than both LN-ISO and LN-SK (TRT*Week, $P = 0.003$; Table 3.9; Figure 3.15). By week 4, LN-ISO had the highest latency to retreat percentage among all treatment groups (TRT*Week, $P = 0.003$; Table 3.9; Figure 3.15).

Dominance Indices

The pen dominance index quantified how unequal the social contest was between pen mates, providing insight into whether dominance was clearly established or more evenly distributed. Higher values (closer to 100%) indicated that one pig consistently performed more advances and displacements, dominating its pen mate and suggesting a more asymmetrical social relationship. Values close to 50% reflected a more balanced dynamic, where both pigs engaged in agonistic behaviors at similar frequencies, indicating mutual social interaction without a clearly dominant individual. Lower values may indicate that limited contest behaviors occurred between pen mates. OBWL-SK showed a consistent reduction in pen dominance index percent, with values significantly lower than their week -2 value at all subsequent time points (weeks 1, 2, 4, and 6; TRT*Week, $P = 0.001$; Table 3.9; Figure 3.16). In contrast, OBWL-ISO only exhibited a reduction from their week -2 value, at week 1 (TRT*Week, $P = 0.001$; Table 3.9; Figure 3.16). Between-group comparisons at week 1, OBWL-ISO revealed a lower pen dominance index percent than LN-ISO and LN-SK (TRT*Week, $P = 0.001$; Table 3.9; Figure 3.16). At week 2,

the lowest pen dominance index percent was observed in OBWL-SK (TRT*Week, $P = 0.001$; Table 3.9; Figure 3.16). By week 4, LN-SK had the highest pen dominance index percent (TRT*Week, $P = 0.001$; Table 3.9; Figure 3.16), and at week 6, the same group maintained a higher percentage compared to OBWL-ISO (TRT*Week, $P = 0.001$; Table 3.9; Figure 3.16).

The individual dominance index quantified how dominant a single pig was within its pen. Since dominance is distributed between two pigs in each pen, the average of the individual dominance index typically does not exceed 50%. Higher individual dominance index values (closer to 50%) indicated that one pig clearly dominated its pen mate, while lower values suggested more balanced interactions or a less assertive expression of dominance. OBWL-SK showed significant decreases in individual dominance index percent from their week -2 value, at all the subsequent weeks (weeks 1, 2, 4, and 6 (TRT*Week, $P = 0.048$; Table 3.9; Figure 3.17). In comparison, OBWL-ISO exhibited a reduction only at weeks 4 and 6 (TRT*Week, $P = 0.048$; Table 3.9; Figure 3.17), while LN-ISO decreased its individual dominance index percent only at week 4 (TRT*Week, $P = 0.048$; Table 3.9; Figure 3.17). At week 1, LN-ISO and LN-SK showed a higher individual dominance index percent than OBWL-ISO and OBWL-SK (TRT*Week, $P = 0.048$; Table 3.9; Figure 3.17). At week 2, OBWL-SK had a lower individual dominance index percent than both OBWL-ISO and LN-SK (TRT*Week, $P = 0.048$; Table 3.9; Figure 3.17). At week 4, LN-SK had a higher individual dominance index percent compared to OBWL-SK (TRT*Week, $P = 0.048$; Table 3.9; Figure 3.17).

Cognitive Task Engagement During Live Observation: Paddle Presses and Contrafreeloading

As described before, paddle press behavior was quantified using data from live observations during the feeder cognitive test. Each occurrence was classified into one of three

categories: (1) both correct paddles were pressed, indicating that one of the pigs did all the correct work; (2) one correct paddle was pressed, suggesting equal contribution on correct work between pen mates; or (3) no correct paddle was pressed, one of the pigs did not do any correct work.

LN-SK and OBWL-ISO had a greater number of observations where a pen mate pushed both correct paddles than the LN-ISO and OBWL-SK after liquid supplementation was provided (Figure 3.18; $\chi^2(df=3, n = 1206) = 14.84; P = 0.002$). OBWL-SK had the most reduction (65%) of pressing both correct paddles after the liquid supplementation was applied (Figure 3.18; $\chi^2(df=3, n = 1206) = 14.84; P = 0.002$). Subsequently, LN-SK had the lowest observations of pressing no correct paddles after liquid supplementation was applied (Figure 3.19; $\chi^2(df=3, n = 599) = 1.1318; P = 0.015$). After supplementation, all the pigs that received sorghum fermented kombucha had a 22% reduction in observations of pressing both correct paddles, whereas all pigs in the isocaloric treatment had a 12% reduction (Figure 3.20; $\chi^2(df=1, n = 1036) = 22.57; P < 0.001$).

LN-SK submissive pigs were 5 times more likely to press both correct paddles than their LN-SK dominant pen mates (Figure 3.21; $\chi^2(df=3, n = 387) = 38.0036; P < 0.001$). Out of the dominant pigs, OBWL-ISO pigs pressed both correct paddles the most, and out of the submissive pigs, LN-SK pressed both correct paddles the most (Figure 3.21; $\chi^2(df=3, n = 387) = 38.0036; P < 0.001$). Consequently, LN-ISO dominant pigs were 8 times more likely not to press a correct paddle than their LN-ISO submissive pen mates. Among the dominant pigs, LN-ISO did not press any correct paddle the most (Figure 3.22; $\chi^2(df=3, n = 144) = 8.94; P < 0.030$).

Discussion

Feeding, Drinking, and Jumping Behavior

All animals in the current experiment decreased the eating percentage duration (length of eating occurrence time) over the sample periods, regardless of receiving isocaloric or sorghum kombucha supplemental liquids. This finding suggests that time typically allocated to feed consumption may have shifted toward the intake of the provided liquid supplements. Likely reflects an adjustment in feeding priorities or time budgeting in response to the new dietary elements (Piles et al., 2025). In contrast, drink percentage duration remained relatively the same for most groups, except for OBWL-ISO, which demonstrated a marked increase in drink duration, particularly at weeks 2 and 4. In addition, the frequency of drinking (number of occurrences or visits to the drink bowls) among this cohort declined at week 4 but remained high across the other weeks, suggesting that the OBWL-ISO pigs may have consumed the liquid supplementation more gradually and persistently, possibly taking smaller sips during each visit. While a lower frequency may reflect the consolidation of intake into longer bouts, a higher frequency can indicate increased arousal, motivation, or interest in the supplementation, or even anxiety (Kirkden and Pajor, 2006). The decreased motivation to drink the isocaloric solution and reduced meal consumption exhibited by obese pigs may represent a carryover effect of prolonged exposure to a Western-style diet. This aligns with findings in Yucatan minipigs, where in a two-choice test, prior exposure to a Western diet and subsequent obesity led to reduced high-sucrose feed intake (grams) compared to both their lean counterparts and pigs after undergoing a weight loss process (Gautier et al., 2020). Gautier et al. (2020) concluded that the amount of high-sucrose feed consumed, together with increased “bite” and “sniff” frequencies, was an indicator of feeding motivation and meal structure, with obese minipigs consuming less feed but

displaying a snacking-type eating pattern. Similarly, in male Sprague Dawley rats transitioning from a western diet to a standard diet, reduced food intake was observed immediately following the diet change (South et al., 2012). For the current experiment, it appears that the isocaloric obese pigs may have had more behavioral carryover from the western diet than the sorghum kombucha obese pigs.

By week 6, only the OBWL-SK maintained lower frequencies of feed bowl switching, while all other treatment groups exhibited increased switching behavior. Bowl switching can serve as a proxy for contest or resource monitoring behaviors, especially in socially housed animals where access to resources may be perceived as limited or competitive (Hsia and Wood-Gush, 1983). Therefore, the stability observed in the kombucha-treated obese pigs may reflect reduced social tension or diminished competitive motivation (De Jonge et al., 2008; Gautier et al., 2020). Collectively, these findings raise the possibility that fermented sorghum kombucha may promote behavioral regulation, either by influencing satiety or by reducing the perceived need for competition. While Gautier et al. (2020) did not explicitly link bowl switching to affective stability, their data on switch time suggest that changes in this behavior may be related to motivation and feeding patterns, which could, in some contexts, influence the consistency of food choices. In their study, pigs were single-housed, so switching reflected only individual feeding decisions without social competition. In a pair-housed context, however, reduced switching could also indicate avoidance of a pen mate at a given bowl, or a decisive preference maintained despite potential competition, whereas increased switching might reflect competitive checking of both bowls.

Similarly, only OBWL-SK exhibited lower drinking frequency compared to all other treatment groups at multiple post-supplementation weeks. Concerning the eat-to-drink interval,

this group demonstrated a reduction by week 6, signifying a more rapid shift from solid to liquid intake. This behavioral change may indicate a heightened incentive or preference for fermented kombucha over a solid meal. Supporting this interpretation, Gautier et al. (2020) reported that diet-induced obese Yucatan pigs, following a weight loss regimen, reverted to preferring the high-energy diet they had previously consumed instead of choosing standard chow, highlighting how previous diet history can influence post-restriction preferences. This parallels findings in human research, where individuals undergoing GLP-1 receptor agonist treatment report reduced desire for sweet or fatty foods and increased preference for sour, fermented, or complex flavors (Van Bloemendaal et al., 2014; Van Can et al., 2014; Bettadapura et al., 2025). These changes may reflect altered palatability or reward sensitivity (De Jonge et al., 2008). Supporting these findings, the same treatment group (OBWL-SK), exhibited the shortest latency to drink at weeks 4 and 6, suggesting they were quicker to initiate drinking compared to all other groups. These combined patterns, fewer but faster-initiated drinking bouts, could corroborate a behavioral preference for the fermented sorghum kombucha over food. Notably, Nelson et al. (2023) also observed shorter latencies to liquid consumption were also observed in pigs offered sorghum kombucha over an isocaloric solution and water.

Latency to engage with a reward is commonly interpreted as an index of motivation (Elizabeth Bolhuis et al., 2013; Melotti et al., 2013). However, when considered alongside low bowl switching patterns and efficient drinking behavior, these findings may also reflect the involvement of working memory. Working memory enables animals to retain and act upon information about previously visited or rewarded locations, thereby reducing unnecessary exploratory behavior (Held et al., 2001; Bolhuis et al., 2013). In pigs, enhanced working memory

performance has been associated with faster decision-making and fewer repeated visits to unrewarded sites in spatial or foraging tasks (Allen et al., 2023).

Although jumping behavior was only expressed by a subset of pigs, our preliminary analysis suggests it may reflect a specific behavioral preference for fermented sorghum kombucha. In this study, jumping occurred in a contextually predictable pattern, consistently near the feeder and immediately prior to liquid supplementation access, indicating it was not a random or stereotypic behavior, but one tied to reward anticipation. Pigs receiving fermented sorghum kombucha exhibited increased jumping, whereas those provided the isocaloric solution showed reduced or extinguished jumping, reinforcing the kombucha's affective relevance. Referral behaviors, like the jump on this contest, indicate actions that direct another individual's attention to a specific object or goal, and have been widely described in human-animal interactions, particularly in dogs. For example, dogs often use gaze alternation or physical gestures to request human assistance or access to desired items (Siniscalchi et al., 2018; Bhattacharjee et al., 2020). Possibly, the consistent jumping observed in this study serves a similar referential function, allowing pigs to signal their anticipation or desire to the human delivering the kombucha. Zupan et al. (2016) reported that pigs that frequently interacted with humans exhibited a greater propensity to explore and engage in play inside unfamiliar situations, indicating that affirmative human interactions augment pigs' affective expression and behavioral range. Taken together, the context, consistency, and communicative nature of the jumping behavior observed here raise the possibility that some pigs may use functionally referential behaviors.

OBWL-ISO exhibited a reduction in non-nutritive oral behavior (NNOB) percentage duration at weeks 2 and 4. Similarly, Gautier et al. (2020) found that Yucatan minipigs

undergoing a three-week weight loss period showed shorter NNOB durations than obese counterparts maintained on a Western diet. In contrast, the OBWL-SK was the only treatment group to show an increase in NNOB percentage duration by week 6. By comparison, Nelson's (2023) earlier observations showed that lean adult Ossabaw pigs had greater NNOB duration towards the isocaloric and water solution than towards the fermented sorghum kombucha during a 3-way preference test. In the present experiment, NNOB latencies were generally high, most likely occurring once nutritive behaviors had been completed. In this context, NNOB are interpreted as exploratory behaviors, particularly given that pigs were housed in enriched pens. Similar patterns have been described in the Human Approach Test with Göttingen minipigs kept in enriched environments (Hulbert et al., 2019). Hulbert et al. (2019) reported that pair-housed minipigs spent a greater percentage of time performing NNOB compared with individually housed counterparts, with oral manipulation of pen fixtures, enrichment items, and other surfaces being a common part of environmental investigation. Ossabaw pigs are feral-derived and possess strong natural foraging and persistence behaviors, unlike conventional domestic breeds. Therefore, increased NNOB in this context may reflect functional, goal-oriented behavior rather than stereotypy or stress. In other words, what appears as non-nutritive oral behavior may actually be a natural expression of foraging motivation in this model.

Cognitive Behavior

Prior to the introduction of any liquid supplements, all obese pigs exhibited longer latencies to press the correct paddle compared to all lean pigs. These results align with previous findings from the same animals at the same project, in which obese pigs demonstrated traditional indicators of cognitive deficits in both working and long-term memory, as assessed using an 8-radial maze (Scott, 2025). Similarly, Gautier et al. (2020) found that Yucatan minipigs

maintained on a high-calorie, high-fat, and high-sucrose diet displayed impaired working memory compared to lean controls when evaluated in a hole board task. Interestingly, by week 4 of the current study, obese pigs on a weight-loss program that received fermented sorghum kombucha exhibited latencies to press the correct paddle comparable to those of lean pigs receiving the isocaloric control, suggesting a potential cognitive benefit of the kombucha supplementation. In contrast, lean pigs receiving fermented sorghum kombucha did not show improvement at that same time point. Other metrics of working memory, such as repeated correct paddle presses, did not yield significant differences, likely due to a floor effect, as discussed by Martin and Bateson (2007), resulting from the overall high learning rates among pigs. To detect more subtle cognitive shifts, future studies should consider a finer sampling resolution, ideally capturing behavior at every feeding session, potentially using AI-based tracking tools similar to those employed in the 8-radial maze study by Scott (2025). In this study, latency to eat, which was contingent upon correctly pressing the paddle, can also be interpreted as a cognitive measure. This variable followed a pattern similar to that of latency to the correct paddle press, with obese pigs displaying the longest delays prior to any liquid supplementation but aligned with the other groups in the later weeks.

Agonistic Interactions During Feeding Cognitive Test

Agonistic behavior is a central component of social interactions in domestic animals, particularly pigs. As defined by McGlone (1986), this behavior category includes a spectrum of behaviors ranging from threats to overt aggression and submission. Scott and Fredericson (1951) originally defined agonistic behavior as the full suite of actions associated with fighting, including attacking, defending, fleeing, and appeasement. These elements typically unfold in a sequence during social conflict, especially in newly formed groups of animals, as also shown by

McGlone (1985). According to Adams (1979), the expression of agonistic behavior is influenced by multiple factors, including physiological state, environmental stressors, and social context. In the current study, agonistic behavior includes both retreats and advances, capturing the full range of social interactions occurring during the feeder test.

Pigs in the LN-SK treatment exhibited increased frequency (number of occurrences) and percentage duration (length of time) of agonistic interactions at weeks 4 and 6, whereas OBWL-SK maintained or decreased their levels of agonism, especially the agonistic duration. These findings align with Schnebel and Griswold (1983) observation that agonistic interactions in pigs are influenced more by resource motivation than by physical aspects alone. The increased agonism observed in LN-SK may reflect heightened motivation to access or control feeder resources. In contrast, the lower agonism observed in obese pigs may result from reduced physical engagement or altered satiety regulation. In rodent models, green and black tea kombucha consumption has been linked to increased gut propionate production, a short-chain fatty acid known to stimulate GLP-1 secretion via activation of FFAR2 (Free Fatty Acid Receptor 2) on enteroendocrine L-cells, which supports satiety signaling and may reduce appetite-driven behaviors (Costa et al., 2022). Overall, kombucha may have different effects on obese individuals in a weight loss program and lean individuals.

Latency, defined as the interval between a triggering event and the initial expression of a behavior (Martin and Bateson, 2007), was used in this study to assess hesitation or deliberation prior to the initiation of agonistic interactions. Latency to initiate agonistic activity increased, especially in the last weeks (weeks 4 and 6), in all treatments. Longer latencies, as noted by Gieling et al. (2011), may suggest that these animals have become more strategic in their social interactions or that they have exhibited reduced impulsivity and greater cognitive control.

In this study, latency to retreat was interpreted as an indicator of submission, particularly given the context of dyadic interactions at the feeder. Retreat behavior alone has been consistently described as a submissive response in pigs, often displayed by subordinate individuals in response to competitive or aggressive threats (Schnebel and Griswold, 1983; Camerlink et al., 2016; Camerlink et al., 2017). However, a longer latency to retreat may suggest a greater willingness to engage in social competition. Lean pigs showed progressively increased latency to retreat. Interestingly, LN-ISO had higher latency to retreat in week 4. These may indicate an intensified urge to secure access to valued resources, such as feed or liquid supplementation. These pigs might be trying to keep access by delaying their withdrawal from the resources (Schnebel and Griswold, 1983; McGlone, 1985).

Dominance Indices

The dominance index is a measure of the relationship equity between a pair of pigs based on wins and losses (Büttner et al., 2019). A pen dominance index of 50% represents moderate engagement in dominance-related behaviors within a dyadic setting, while a score approaching 100% reflects a pig that consistently initiates and succeeds in displacing its pen mate, indicating a clearly dominant status within the pair. In contrast, a pen with a dominance index below 50% likely represents a dyad where both pigs exhibited low levels of competitive behavior, suggesting limited social hierarchy formation or reduced motivation to assert dominance (Büttner et al., 2019). In this study, the individual dominance index was calculated using the average of the percentage of advances initiated and the percentage of displacements inflicted on the pen mate. As only one pig in a dyad typically assumes the dominant role, and the other displays submissive tendencies, the individual index naturally tends to remain below 50% when averaged across all pigs. This is especially evident in pens where

dominance is not strongly polarized or when one member of the dyad exhibits low reactivity or withdrawal (Oldham et al., 2020). Büttner et al. (2019) showed that such outcome-based dominance metrics are strongly correlated with network-derived centrality measures, supporting their validity in capturing social hierarchy structure.

OBWL-SK exhibited consistent reductions in both dominance indices over time, whereas their isocaloric counterparts showed a decline in dominance indices at week 1, but then increased at week 2. This finding suggests that sorghum kombucha attenuated their social competitiveness. Turner et al. (2001) observed in group-housed growing pigs (Large White x Landrace) fed ad libitum that pigs of lower relative body weight displayed fewer dominant behaviors, supporting the idea that body condition may influence social status and feeding dynamics. Comparable findings were described in greater long-tailed hamsters (*Tscherskia triton*), where dominant status was associated with heavier body weight and more frequent agonistic behaviors (Pan et al., 2010). These cross-species comparisons reinforce the idea that both metabolic status and perceived social opportunity shape dominance dynamics in social mammals. Although in the current experiment, both obese groups were on a weight loss program, the pronounced drop in dominance indices at week 2 was only observed in the fermented sorghum kombucha group. This divergence suggests that the effect was not solely due to body condition but may reflect a specific modulatory role of kombucha on social behavior.

While obese pigs' relationships may have benefited from sorghum kombucha, lean pigs supplemented with fermented sorghum kombucha exhibited greater pen-level dominance at week 4 compared to obese pigs on a weight loss program within the same treatment. This indicates that without a change in diet, supplementation may cause more competition behaviors or elevate arousal and activity, thereby enhancing social standing. These findings can be related to their

palatability for fermented sorghum kombucha, as documented by Nelson et al. (2023), who showed that adult lean Ossabaw pigs have a high affinity for fermented sorghum tea over isocaloric solutions. This greater preference may have boosted competition among pigs for the solution, resulting in more dominant social behaviors in the lean kombucha group.

Cognitive Task Engagement During Live Observation: Paddle Presses and Contrafreeloading

The current study provides clear evidence of contrafreeloading behavior during the feeder-based cognitive test, as characterized by pigs engaging in effortful paddle pressing (pressed both correct paddles rather than waiting for penmate to press both paddles). Aligned with previous work on contrafreeloading, which has been observed in a wide range of species, where animals willingly choose work for a reward over consuming freely available alternatives (Young and Lawrence, 2003; De Jonge et al., 2008; Lindqvist and Jensen, 2008; McGowan et al., 2010; Van Zeeland et al., 2023). Submissive pigs were more likely to engage in contrafreeloading behavior, while dominant pigs often avoided the cognitive task and instead exhibited freeloading strategies (no paddle pressed). A similar pattern was reported by Nelson (2024), who observed adult Ossabaw pigs in a comparable feeder-based cognitive test while validating the same CogShape feeder as a novel behavioral assessment tool.

Interestingly, fermented sorghum kombucha appeared to intensify social and feeding competition among lean pigs, as reflected in the higher occurrence of contrafreeloading behavior among submissive individuals within this group. In addition, this group showed the greatest reduction in the number of pigs that failed to engage in the task following the introduction of the liquid supplementation. This pattern may suggest a potential physiological influence on reward-related pathways (Berridge and Robinson, 2003; Knutson and Cooper, 2005). However, further

investigation, including brain histology and molecular profiling, is necessary to confirm any health-related or neurological mechanisms.

When comparing the proportion of pigs performing all the cognitive tasks (pressing both correct paddles) before and after liquid supplementation, obese pigs receiving sorghum kombucha appeared to benefit behaviorally, showing the greatest reduction in instances where only one pig completed the task, aligning more closely with the values observed in the LN-ISO group. This change may reflect a decrease in intra-pair competition, suggesting more balanced cognitive engagement between pen mates. Overall, the result indicates a shift in social dynamics. Such shifts are consistent with the notion that dominance status is not static and can be modulated over time by physiological or environmental factors (Meese and Ewbank, 1973; McGlone, 1986; O’Connell and Beattie, 1999).

Recognizing that social interactions significantly influenced behavioral responses, this aspect of the study highlights an important consideration. Many biological models rely on single-housed animals, which inherently limits the ability to assess social or cooperative behaviors (NRC, 2011; Gautier et al., 2020).

Conclusions

The cognitive feeder test revealed that fermented sorghum kombucha influenced feeding and social behaviors in both lean and obese Ossabaw pigs, with the most pronounced benefits observed in the obese group, showing reduced motivation to keep a contest happening, and more balanced social interactions between pen mates. Sorghum kombucha supplementation appeared to increase social and feeding competition among lean pigs, suggesting a possible modulation of motivational or reward-related pathways. Preference for kombucha was evident across groups; both lean and obese pigs displayed a stronger behavioral response compared to those receiving

the isocaloric solution. These findings support the potential of fermented sorghum kombucha as a behavioral modulator in socially housed pigs. However, further research is needed to explore the underlying physiological mechanisms and the long-term persistence of these effects beyond the early stages of dietary transition.

Implications

The current study supports the broader objective of evaluating whether fermented sorghum kombucha can serve as a dual-purpose intervention, both physiological and behavioral, for managing obesity. In addition to previous results showing that fermented sorghum kombucha may reduce average daily gain and greater back fat percentage change accumulation in obese pigs, this chapter demonstrates that kombucha also influences social behavior, feeding patterns, and cognitive engagement. The noted behavioral alterations indicate that fermented kombucha may assist obese individuals in achieving greater behavioral flexibility and reduced reactivity to feeding-related stressors. These results emphasize the potential of sorghum kombucha as a dietary component that can influence motivation and social interaction behaviors in obesity, in addition to its ability to facilitate weight management. As behavioral dysregulation plays a key role in the development and persistence of obesity, fermented sorghum kombucha may represent a novel nutritional strategy to support both metabolic health and behavioral adaptation to the stress of being on a weight loss diet and period.

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Tables

Table 3.1 Primary ethogram (subject actions) used on Noldus Observer XT for manual coding.

Behavior	Definition	Classification	Code
Social Feeding			
Advanced	Pig moves toward the other pig	Event	+
Retreat	Pig moves away from the other pig after an interaction	Event	-
Head still	Pig remains with head still, no clear approach or avoidance, all four legs at the floor	State	0
NNOB ¹	Pig is sniffing around the feeder area or any other non-nutritive oral behaviors ¹	State	1
Jump up	Pigs jumps up and contacts with the two front forelimbs on top of the feeder	Event	7
Exit the feeder area	Pig exits the feeder area, both ears outside of the feeder area	Event	.
Feeding and Drinking			
Left feed bowl	Pig consumes feed from the left food bowl, both ears inside of the feed bowl	State	8
Right feed bowl	Pig consumes feed from the right food bowl, both ears inside of the feed bowl	State	9
Left tea ² bowl	Pig drinks from the left tea ² bowl, both ears inside of the drink bowl	State	/
Right tea ² bowl	Pig drinks from the right tea ² bowl, both ears inside of the drink bowl	State	*
Cognitive test			
Left correct paddle	Pig presses the left paddle that releases food with snout, head or forelimbs	Event	4
Right correct paddle	Pig presses the right paddle that releases food with snout, head or forelimbs	Event	2
Left incorrect paddle	Pig presses the left paddle that does not release snout, head or forelimbs	Event	5
Right incorrect paddle	Pig presses the right paddle that does not release snout, head or forelimbs	Event	3

¹Non-nutritive oral behaviors include licking, chewing, chomping, rubbing, rooting, and sniffing (including jump up and sniffing) using the mouth, snout, or face (adapted from Hulbert et al., 2019). ²Tea refers to the liquid supplementation (isocaloric solution or sorghum kombucha).

Table 3.2 Secondary ethogram (feeder and human actions) used on Noldus Observer XT for manual coding.

Behavior	Definition	Classification	Code
Feeder light			
Left feed light on	Light start blinks at the left side of feeder	Event	i
Right feed light on	Light start blinks at the right side of feeder	Event	l
Left feed light off	Light stop blinks at the left side of feeder	Event	j
Right feed light off	Light stop blinks at the right side of feeder	Event	k
Both lights off	Both lights go off	State	o
Human actions			
Funnel right tea ¹ bowl	Human pours tea ¹ with the funnel into the right tea bowl	Event	b
Funnel left tea ¹ bowl	Human pours tea ¹ with the funnel into the left tea bowl	Event	c
Funnel right feed bowl	Human pours feed with the funnel into the right feed bowl	Event	v
Funnel left feed bowl	Human pours feed with the funnel into the left feed bowl	Event	x
No Funnel	No funnel into the feeder area	State	z

¹Tea refers to the liquid supplementation (isocaloric solution or sorghum kombucha).

Table 3.3 Duration (% of observation) of behaviors measured across five weeks in lean (LN) and obese on a weight loss program (OBWL) pigs (n = 32), receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK).

Duration, % of obs		LN		OBWL		Lgst SEM	P-value TRT*Time
		ISO	SK	ISO	SK		
<i>Outside feeder area</i>	Week -2	0.8	2.6	2.4	3.4	1.40	0.8044
	Week 1	1.4	3.2	0.4	1.8	1.03	
	Week 2	3.2	3.8	5.7	2.9	1.63	
	Week 4	3.7	4.8	4.7	2.4	1.80	
	Week 6	4.1	6.2	5.4	2.2	2.50	
<i>Inside feeder</i>	Week -2	99.2	97.4	97.6	96.6	1.37	0.8044
	Week 1	98.6	97.0	99.6	98.0	1.08	
	Week 2	96.8	96.4	94.8	97.2	1.65	
	Week 4	96.3	95.4	95.8	97.7	1.85	
	Week 6	95.9	94.2	95.3	97.9	2.55	
<i>Eat</i>	Week -2	86.0	88.3	84.3	82.9	2.55	0.0001
	Week 1	81.8 ^{ac}	76.4 ^{†a}	90.9 ^{†b}	85.1 ^{bc}	2.43	
	Week 2	73.2 [†]	69.1 [†]	64.8 [†]	73.2 [†]	3.33	
	Week 4	76.9 ^{†ac}	71.7 ^{†ab}	68.5 ^{†b}	78.4 ^c	2.46	
	Week 6	76.4 ^{†ab}	80.9 ^{†a}	70.8 ^{†b}	79.6 ^a	2.51	
<i>NNOB¹</i>	Week -2	9.2	9.9	13.0	9.4	2.06	0.0194
	Week 1	7.4	8.9	10.6	9.6	2.07	
	Week 2	11.2	9.6	6.0 [†]	6.2	2.06	
	Week 4	8.5	8.9	5.7 [†]	7.3	2.06	
	Week 6	10.4	10.6	12.9	14.2 [†]	2.06	
<i>Drink</i>	-	-	-	-	-	-	0.0005
	Week 1	8.2 ^a	9.6 ^a	2.1 ^b	5.0 ^{ab}	1.67	
	Week 2	10.1 ^{ab}	10.7 ^{ab}	14.8 ^{†a}	9.2 ^b	1.80	
	Week 4	9.1 ^a	9.0 ^a	14.8 ^{†b}	8.0 ^a	1.68	
	Week 6	9.0	9.4	11.0 [†]	8.3	1.55	
<i>Head Still</i>	Week -2	3.6	3.7	2.6	4.2	0.82	0.4854
	Week 1	4.0	3.2	3.0	3.7	1.07	
	Week 2	1.5	2.1	1.5	1.6	0.51	
	Week 4	2.2	2.5	2.3	3.1	0.53	
	Week 6	2.3	3.5	3.4	4.8	0.65	

^{abc}LS means differ ($P < 0.05$; Tukey's HSD) within week. [†]Within a treatment, LS means differ from week -2 or week 1 for drink ($P < 0.05$; Tukey's HSD). Lgst refers to the largest SEM value observed across treatment groups for that week. ¹Non-nutritive oral behaviors include licking, chewing, chomping, rubbing, rooting, and sniffing (including jump up and sniffing) using the mouth, snout, or face (adapted from Hulbert et al., 2019).

Table 3.4 Frequency (no. per observations) of behaviors measured across five weeks in lean (LN) and obese on a weight loss program (OBWL) pigs (n = 32), receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK).

Frequency, no. per obs		LN		OBWL		Lgst SEM	P-value TRT*Time
		ISO	SK	ISO	SK		
<i>Outside feeder area</i>	Week -2	1.1	1.3	1.4	1.2	0.38	0.3971
	Week 1	1.1	0.9	1.5	1.1	0.34	
	Week 2	1.1	0.9	1.4	1.3	0.36	
	Week 4	1.0	1.4	1.4	1.2	0.36	
	Week 6	1.3	1.8	2.1	1.8	0.34	
<i>Inside feeder area</i>	Week -2	0.7	1.1	1.7	1.4	0.73	0.2046
	Week 1	1.0	0.9	1.5	1.1	0.73	
	Week 2	1.0	0.9	1.6	1.1	0.73	
	Week 4	0.8	1.2	1.6	1.1	0.72	
	Week 6	0.7	1.1	1.7	1.8	0.73	
<i>Eat</i>	Week -2	7.3	6.1	8.3	4.6	1.60	0.1736
	Week 1	6.6	7.9	8.4	4.6	1.58	
	Week 2	7.8	8.6	7.1	5.7	1.60	
	Week 4	7.2	8.9	8.7	6.5	1.59	
	Week 6	9.7	10.9	12.0	5.9	1.55	
<i>Feed bowl switching</i>	Week -2	5.5 ^{ab}	4.7 ^{ab}	7.0 ^a	2.8 ^b	1.30	0.0028
	Week 1	5.1 ^{ab}	5.8 ^{ab}	7.0 ^a	2.8 ^b	1.30	
	Week 2	6.1	6.8 [†]	5.6	4.2	1.30	
	Week 4	5.8	7.2 [†]	6.8	5.0	1.30	
	Week 6	8.2 ^{†ab}	8.9 ^{†a}	10.1 ^{†a}	4.1 ^b	1.30	
<i>NNOB¹</i>	Week -2	4.3	2.5	4.3	2.7	0.78	0.5668
	Week 1	4.9	2.5	3.2	2.9	0.75	
	Week 2	3.8	3.2	3.0	2.8	0.83	
	Week 4	3.1	2.9	2.7	2.1	0.65	
	Week 6	3.6	3.2	3.9	3.4	0.70	
<i>Drink</i>	Week 1	3.7 ^a	3 ^a	5.5 ^b	2.1 ^a	0.50	0.0143
	Week 2	4.9 ^{†a}	4.1 ^{†a}	5.2 ^a	2.5 ^b	0.55	
	Week 4	4.4 ^a	3.9 ^{†a}	3.9 ^{†a}	2.4 ^b	0.53	
	Week 6	5.1 ^{†a}	4.1 ^{†ab}	5.3 ^a	2.7 ^b	0.51	
<i>Drink bowl switching</i>	Week 1	1.3	0.6	2.1	0.4	0.42	0.0842
	Week 2	2.8	2.1	2.4	1.1	0.45	
	Week 4	2.4	2.2	1.6	1.1	0.41	
	Week 6	3.5	2.6	3.7	1.5	0.45	
<i>Head Still</i>	Week -2	3.4	2.6	3.3	2.8	0.45	0.1708
	Week 1	3.6	3.8	3.1	2.2	0.54	
	Week 2	2.2	3.1	2.4	2.4	0.51	
	Week 4	2.6	2.7	2.4	2.3	0.37	
	Week 6	2.4	4.1	3.7	2.6	0.50	

^{abc}LS means differ ($P < 0.05$; Tukey's HSD) within week. [†]Within a treatment, LS means differ from week -2 or week 1 for drink and drink bowl switching ($P < 0.05$; Tukey's HSD). Lgst refers to the largest SEM value observed across treatment groups for that week. ¹Non-nutritive oral behaviors (adapted from Hulbert et al., 2019)

Table 3.5 Latency and Interval (% of observation) of behaviors measured across five weeks in lean (LN) and obese on a weight loss program (OBWL) pigs (n = 32), receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK).

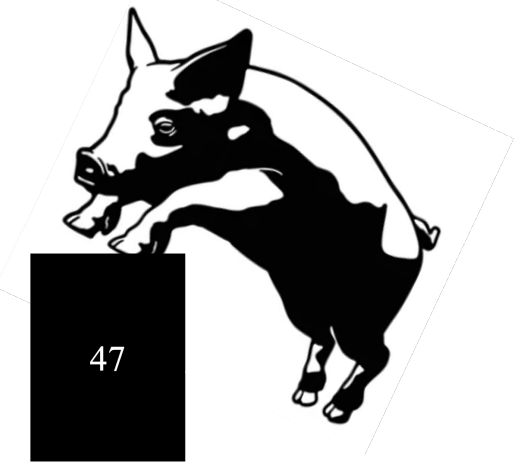
	LN		OBWL		Lgst SEM	P-value TRT*Time
	ISO	SK	ISO	SK		
<i>Latency to NNOB¹, % of obs</i>						0.1438
Week -2	33.8	59.9	21.5	47.5	14.91	
Week 1	47.7	44.1	30.2	49.8	14.91	
Week 2	54.9	69.1	48.3	62.3	14.91	
Week 4	58.8	73.0	61.4	65.4	14.91	
Week 6	53.2	71.2	45.6	56.5	14.91	
<i>Latency to correct paddle², % of obs</i>						0.0161
Week -2	8.9 ^a	11.9 ^a	24.3 ^b	24.6 ^b	4.93	
Week 1	5.6	8.1	0.85 [†]	3.97 [†]	4.93	
Week 2	10.9	13.5	8.75 [†]	8.59 [†]	4.97	
Week 4	0.5 ^{#a}	14.3 ^b	10.1 ^{†ab}	0.8 ^{†a}	4.93	
Week 6	3.7	4.6	0.69 [†]	0.73 [†]	4.93	
<i>Latency to Eat, % of obs</i>						0.0660
Week -2	8.6 ^d	4.5 ^d	7.1 ^d	13.6 ^f	1.71	
Week 1	1.7 ^d	1.2 ^d	1.7 ^d	2.3 ^e	0.21	
Week 2	1.5 ^d	1.5 ^d	2.7 ^d	3.4 ^e	0.53	
Week 4	1.7 ^{de}	1.4 ^e	1.9 ^{df}	2.1 ^f	0.20	
Week 6	1.6	1.8	1.9	2.1	0.23	
<i>Latency to Drink, % of obs</i>						0.0593
Week -2	-	-	-	-	-	
Week 1	51.5	37.4	46.7	48.9	7.63	
Week 2	54.6	52.7 [†]	48.5	48.3	4.79	
Week 4	51.4 ^d	43.6 ^{de}	41.2 ^{de}	34.8 ^{†e}	4.22	
Week 6	47.3 ^{de}	47.9 ^d	44.9 ^{de}	33.9 ^{†e}	4.65	
<i>Eat to Drink Interval, % obs</i>						0.0240
Week -2	-	-	-	-	-	
Week 1	52.5	36.6	44.8	45.7	9.87	
Week 2	50.7	48.5	42.7	33.9	6.43	
Week 4	46.5	42.6	39.5	33.8	6.28	
Week 6	42.5	43.7	43.0	30.3 [†]	6.36	

^{abc} LS means differ ($P < 0.05$; Tukey's HSD) within week. ^{def} LS means show a trend toward difference ($P < 0.10$). [†]Within a treatment, LS means differ from week -2 or week 1 for latency to drink and eat to drink interval ($P < 0.05$; Tukey's HSD). Lgst refers to the largest SEM value observed across treatment groups for that week. ¹ Non-nutritive oral behaviors include licking, chewing, chomping, rubbing, rooting, and sniffing (including jump up and sniffing) using the mouth, snout, or face (adapted from Hulbert et al., 2019). ²A penalty value of 100 was assigned to pigs that did not initiate latency to correct the paddle, % of obs.

Table 3.6 Categorical assessment of lean (LN) pigs and obese pigs on a weight loss program (OBWL) receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK) in relation of motivation to jump before and after liquid supplementation. Chi-square analysis $X^2 (3, N = 85) = 40.87, P < 0.001$.

		Observed		Expected		Residual	
Treatments		Before	After	Before	After	Before	After
LN	ISO	17.00	1.00	6.56	9.52	16.59	9.52
	SK	3.00	26.00	10.58	18.42	5.43	3.12
OBWL	ISO	7.00	6.00	4.74	8.26	1.08	0.62
	SK	4.00	21.00	9.12	15.88	2.87	1.65

Table 3.7 Categorical assessment of pigs receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK) in relation of motivation to jump before and after liquid supplementation. Chi-square analysis $X^2 (1, N=85) = 35.31, P < 0.001$.

	Before	After
ISO	 (11.31)¹ [14.25]²	 (19.69) [8.18]
SK	 (19.69) [8.18]	 (34.31) [4.70]

¹ Expected values of chi-square analysis assuming equal distribution. ² Residual error.

Table 3.8 Duration (% of observations) and frequency (no. per observation) of contest behaviors measured across five weeks in lean (LN) and obese on a weight loss program (OBWL) pigs (n = 32), receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK).

		LN		OBWL		Lgst	P-value
		ISO	SK	ISO	SK	SEM	TRT*Time
<i>Agonistic Duration, % of obs</i>	Week -2	1.8	2.8	2.5	2.3	0.54	0.0001
	Week 1	1.3	0.9 [†]	0.5 [†]	0.6 [†]	0.42	
	Week 2	1.2	1.8 [†]	2.2	1.0 [†]	0.50	
	Week 4	0.9 ^{†ab}	1.9 ^a	0.9 ^{†ab}	0.4 ^{†b}	0.44	
	Week 6	0.8 ^{†ab}	2.4 ^a	1.1 ^{†a}	0.7 ^{†b}	0.41	
<i>Advance Duration, % of obs</i>	Week -2	1.3	1.8	1.8	1.2	0.28	0.1019
	Week 1	0.7	0.5	0.3	0.3	0.20	
	Week 2	0.7	0.9	1.6	0.6	0.32	
	Week 4	0.4	1.1	0.3	0.4	0.30	
	Week 6	0.5	1.0	0.4	0.4	0.25	
<i>Retreat Duration, % of obs</i>	Week -2	0.7	0.9	0.9	0.8	0.26	0.5428
	Week 1	0.6	0.2	0.1	0.3	0.19	
	Week 2	0.6	0.4	0.6	0.2	0.24	
	Week 4	0.6	0.1	0.5	0.1	0.26	
	Week 6	0.6	0.1	0.6	0.25	0.22	
<i>Agonistic Frequency, no. per obs</i>	Week -2	3.1	4.9	4.0	2.5	1.00	0.0001
	Week 1	2.3 [†]	2.1	1.2 [†]	2.4	0.95	
	Week 2	3.9	5.2	4.9	2.4	1.02	
	Week 4	2.2 ^{†a}	6.4 ^b	2.6 ^a	1.9 ^a	0.95	
	Week 6	2.9 ^{ab}	5.7 ^a	3.1 ^{ab}	1.6 ^b	0.94	
<i>Advance Frequency, no. per obs</i>	Week -2	2.2 ^{de}	3.3 ^d	2.8 ^d	1.5 ^e	0.39	0.0519
	Week 1	1.7	1.3 [†]	0.5 [†]	1.1	0.42	
	Week 2	2.2 ^{de}	3.3 ^d	2.8 ^{de}	1.6 ^e	0.56	
	Week 4	1.5 ^{de}	3.3 ^d	1.5 ^{de}	1.3 ^e	0.61	
	Week 6	1.9	2.1	1.7	1.4	0.71	
<i>Retreat Frequency, no. per obs</i>	Week -2	1.4	1.5	1.1	1.2	0.37	0.4708
	Week 1	1.5	0.7	1.7	0.8	0.39	
	Week 2	2.4	1.6	1.1	0.7	0.58	
	Week 4	1.9	1.6	0.2	0.9	0.59	
	Week 6	2.3	0.8	0.1	1.2	0.69	

^{abc} LS means differ ($P < 0.05$; Tukey's HSD) within week. ^{de} LS means show a trend toward difference ($P < 0.10$). [†] Within a treatment, LS means differ from week -2 ($P < 0.05$; Tukey's HSD). Lgst refers to the largest SEM value observed across treatment groups for that week.

Table 3.9 Latency (% of observation) and contest indexes (%) of behaviors measured across five weeks in lean (LN) and obese on a weight loss program (OBWL) pigs (n = 32), receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK).

		LN		OBWL		Lgst SEM	P-value TRT*Time
		ISO	SK	ISO	SK		
<i>Latency to Agonistic, % of obs</i>	Week -2	11.8	9.3	6.8	12.3	5.94	0.0039
	Week 1	31.9 ^{†ab}	40.1 ^{†a}	11.2 ^{ab}	8.3 ^b	12.00	
	Week 2	7.7	19.4	5.9	6.0	8.20	
	Week 4	55.4 ^{†a}	28.8 ^{†b}	20.8 ^b	38.8 ^{†ab}	9.55	
	Week 6	42.3 [†]	36.9 [†]	25.8 [†]	30.9 [†]	9.73	
<i>Latency to Advance, % of obs</i>	Week -2	11.8	10.5	9.1	16.5	5.90	0.3754
	Week 1	30.3	4.8	11.5	31.7	12.90	
	Week 2	23.5	32.0	11.7	9.9	10.94	
	Week 4	46.4	41.2	26.3	46.6	11.74	
	Week 6	39.1	42.7	27.5	40.9	11.87	
<i>Latency to Retreat, % of obs</i>	Week -2	13.2	6.3	14.8	18.4	7.00	0.0028
	Week 1	42.3 ^{†a}	37.2 ^{†a}	14.6 ^{ab}	5.6 ^b	11.12	
	Week 2	12.5	21 [†]	13.9	3.1	9.70	
	Week 4	68.6 ^{†a}	34.6 ^{†b}	17.8 ^b	22.3 ^b	13.86	
	Week 6	52.9 [†]	33.8 [†]	28.2	17.2	16.87	
<i>Individual Dominance Index, %</i>	Week -2	45.2	49.9	50.4	50.4	5.67	0.0485
	Week 1	39.8 ^{ab}	42.3 ^a	18.6 ^{†b}	18.9 ^{†b}	7.58	
	Week 2	37.7 ^{ab}	48.1 ^a	47.2 ^a	21.8 ^{†b}	7.87	
	Week 4	24.9 ^{†ab}	48.2 ^a	38.1 ^{ab}	22.9 ^{†b}	8.39	
	Week 6	26.2	47.6	20.9 [†]	29.1 [†]	9.83	
<i>Pen Dominance Index, %</i>	Week -2	57.9	66.7	64.2	86.9	11.13	0.0011
	Week 1	61.3 ^a	68.7 ^a	22.6 ^{†b}	37.9 ^{†ab}	10.96	
	Week 2	73.2 ^a	78.9 ^a	65.2 ^{ab}	38 ^{†b}	10.95	
	Week 4	47.6 ^{ab}	76.7 ^a	50.8 ^{ab}	45.2 ^{†b}	10.96	
	Week 6	63.7 ^{ab}	74.9 ^a	40.3 ^b	52.8 ^{†ab}	11.13	
<i>Pen Submissive Index, %</i>	Week -2	30.7 ^a	31.8 ^a	33.9 ^a	8.3 ^b	5.85	0.0766
	Week 1	14.9 [†]	16.7 [†]	10.3 [†]	5.25	4.52	
	Week 2	10.1 ^{†ac}	16.7 ^{†ab}	23.9 ^b	5.7 ^c	3.03	
	Week 4	7.2 ^{†a}	13.8 ^{†ab}	21.1 ^{†b}	8.7 ^a	2.98	
	Week 6	4.1 ^{†a}	13.9 ^{†ab}	15.3 ^{†b}	3.8 ^a	3.53	

^{abc} LS means differ ($P < 0.05$; Tukey's HSD) within week. [†]Within a treatment, LS means differ from week -2 ($P < 0.05$; Tukey's HSD). Lgst refers to the largest SEM value observed across treatment groups for that week.

Figures

Figure 3.1 Pen diagram illustrating the layout of the housing environment used during the behavioral study. Subjects were housed in pairs and had unrestricted access to all enrichment items throughout the trial. Each pen included a designated CogShape cognitive feeder (feeder area). The dimensions of the enrichment objects and structures are approximate and not to exact scale.

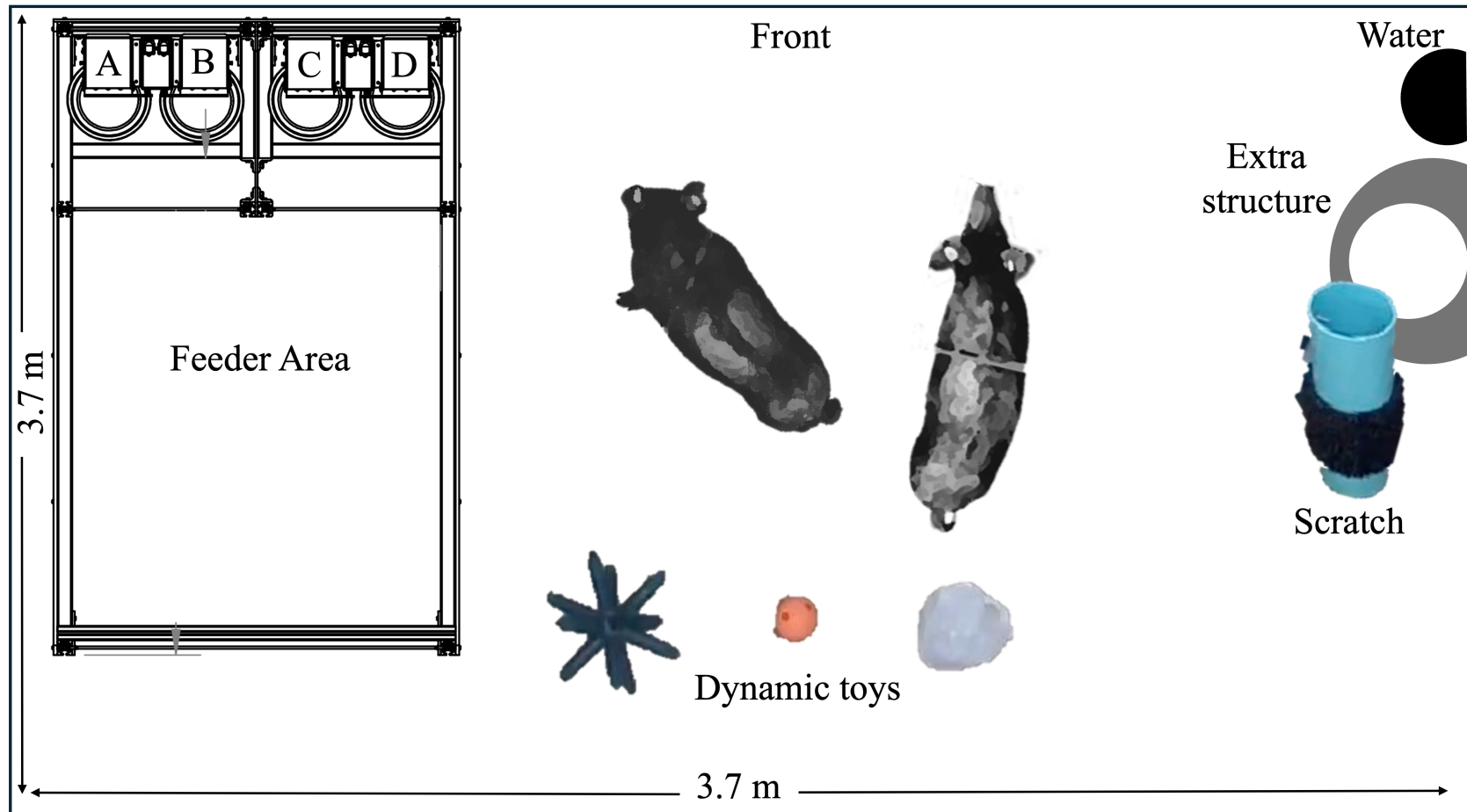


Figure 3.2 Top-view schematic of the cognitive feeder apparatus used in the behavioral study, showing the dimensions. The feeder includes four paddle-activated bowls enclosed within a defined interaction area.

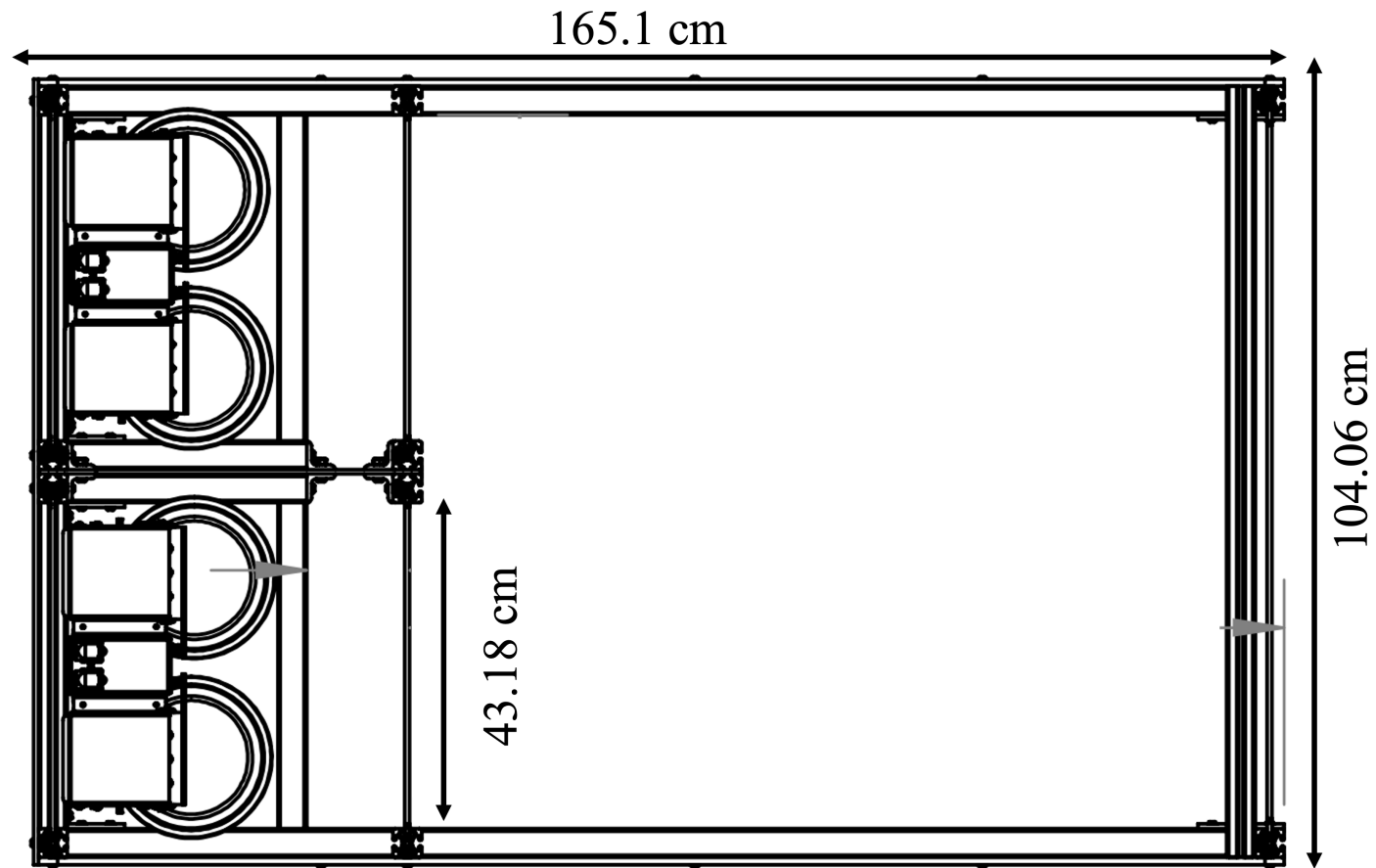


Figure 3.3 General timeline of dietary and feeder cognitive test phases, and harvest age in the study.

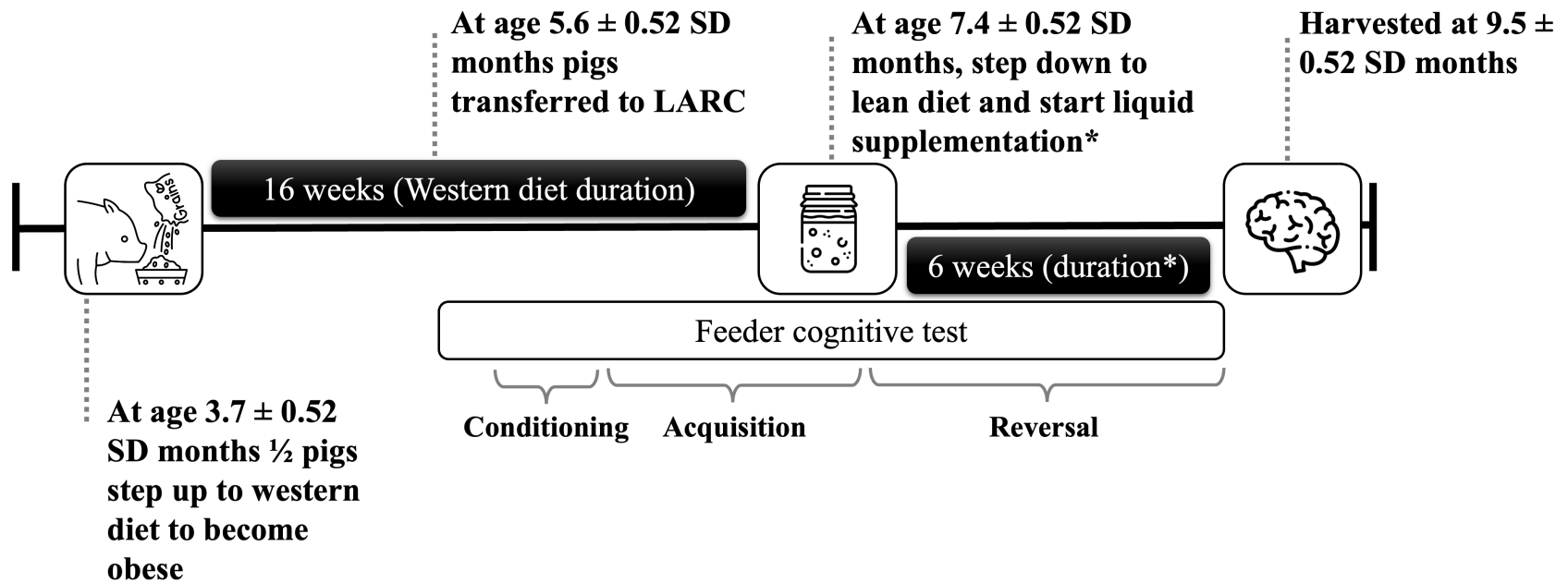


Figure 3.4 Paddle configurations used during the cognitive feeder test in all learning phases.

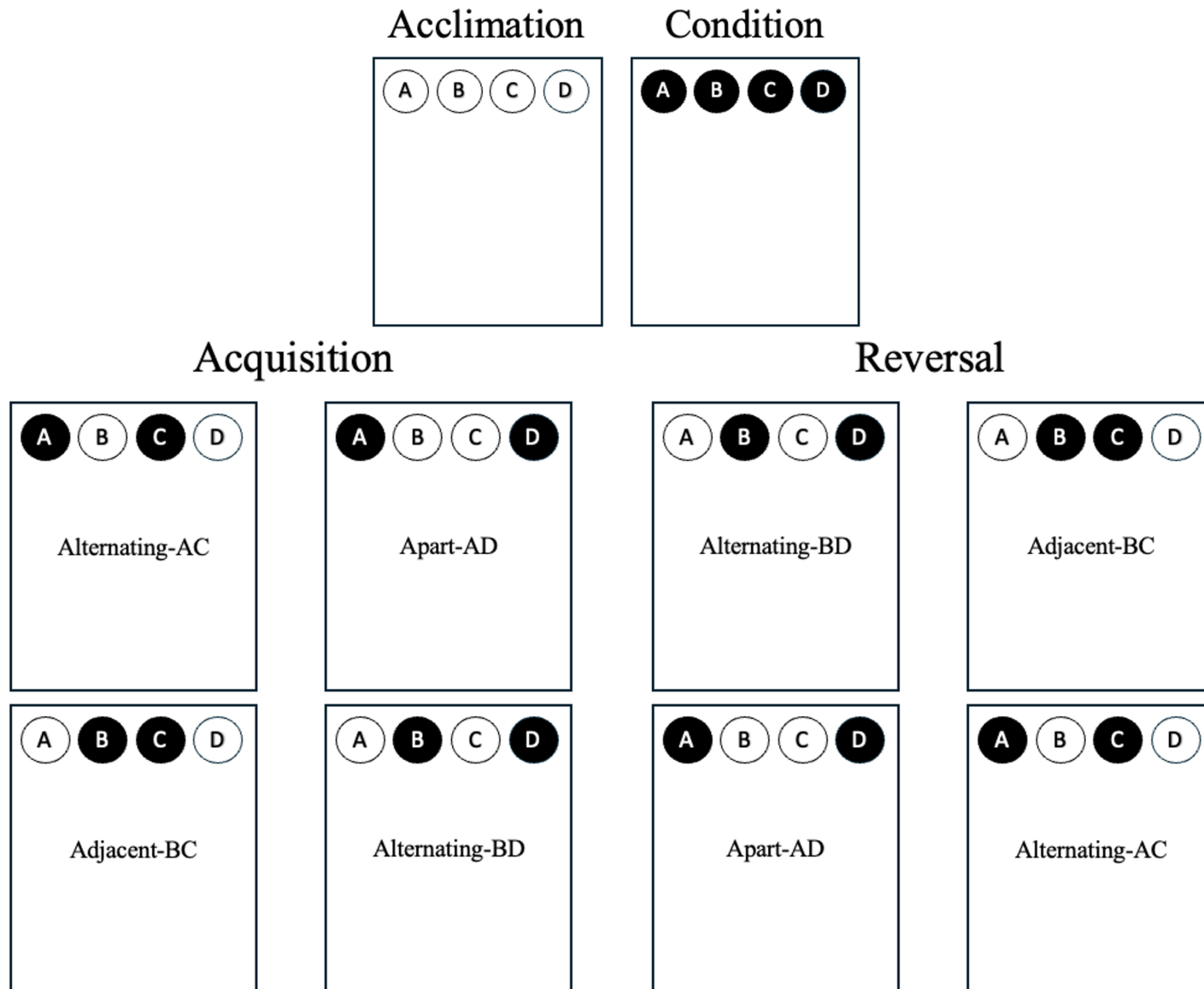


Figure 3.5 Overhead view of the setup illustrating the threshold (dashed line) used to initiate timing for feeder test behaviors.

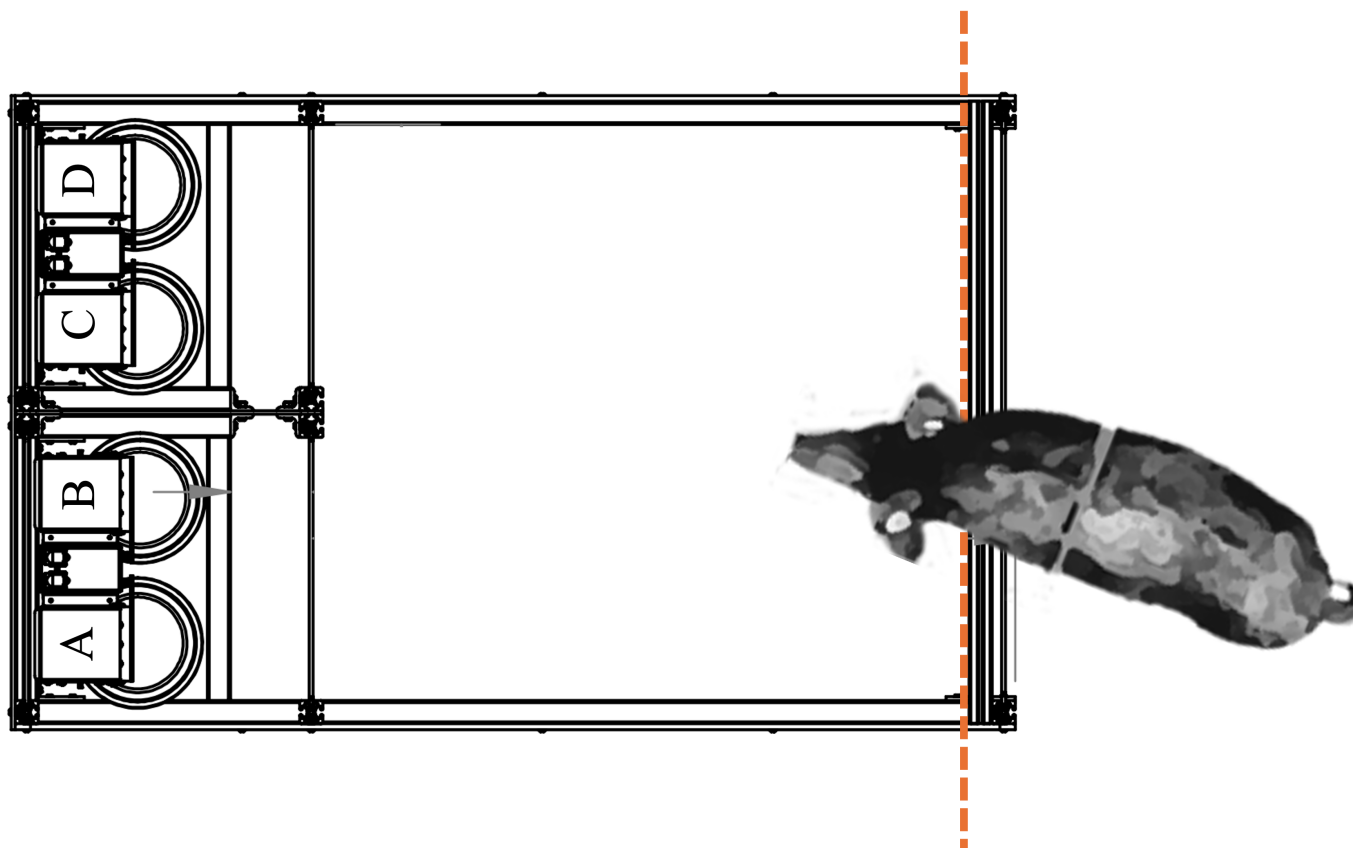


Figure 3.6 Eat duration (% of observations; TRT*Week, $P < 0.001$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *OBWL-ISO differs from LN-ISO and LN-SK; OBWL-SK differs from LN-SK at that time point. **OBWL-ISO differs from OBWL-SK and LN-ISO; OBWL-SK differs from LN-SK at that time point. ***OBWL-ISO had the lowest value at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

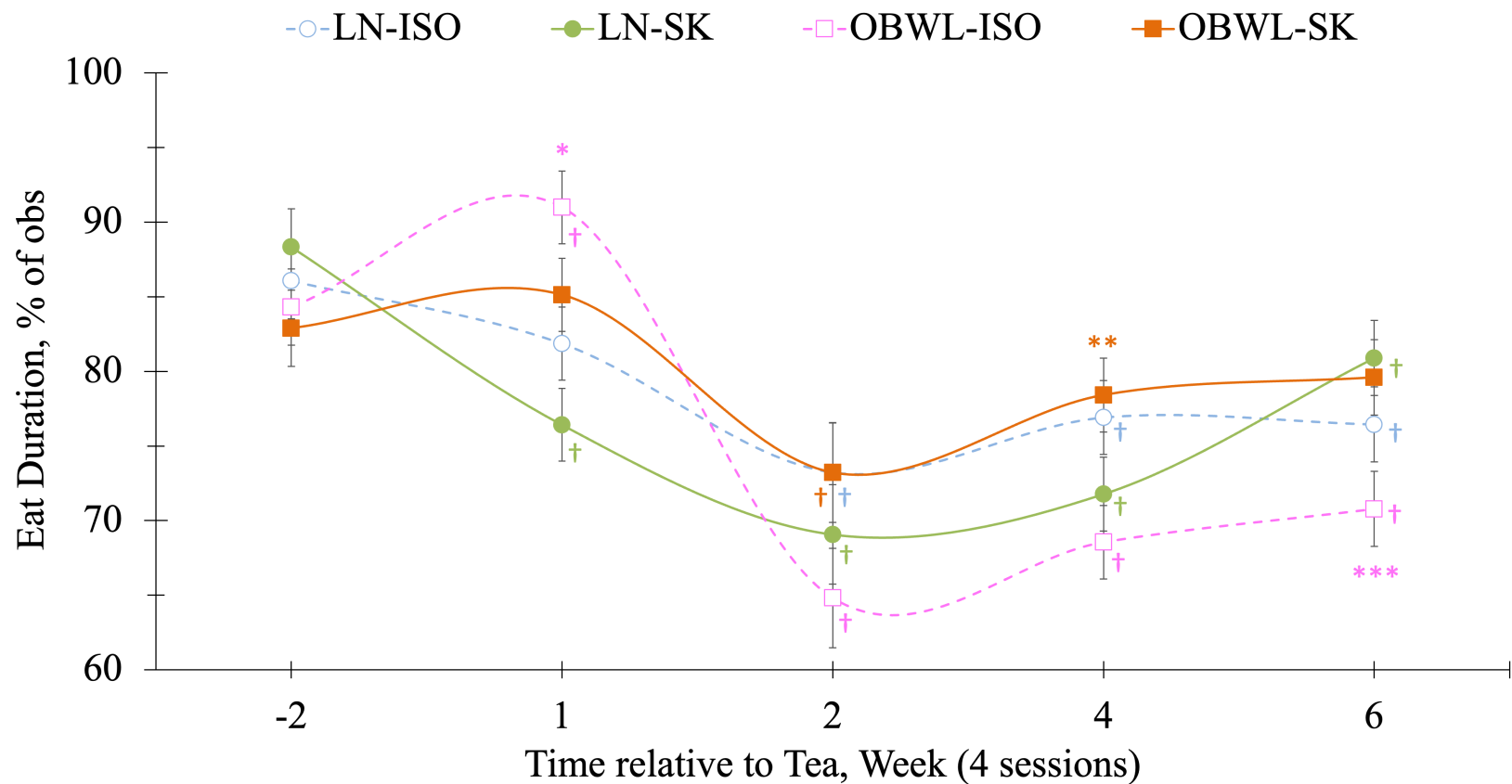


Figure 3.7 Feed bowls switching frequency (number per session; TRT*Week, $P = 0.003$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *OBWL-ISO differs from OBWL-SK at that time point. **OBWL-SK had the lowest value at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

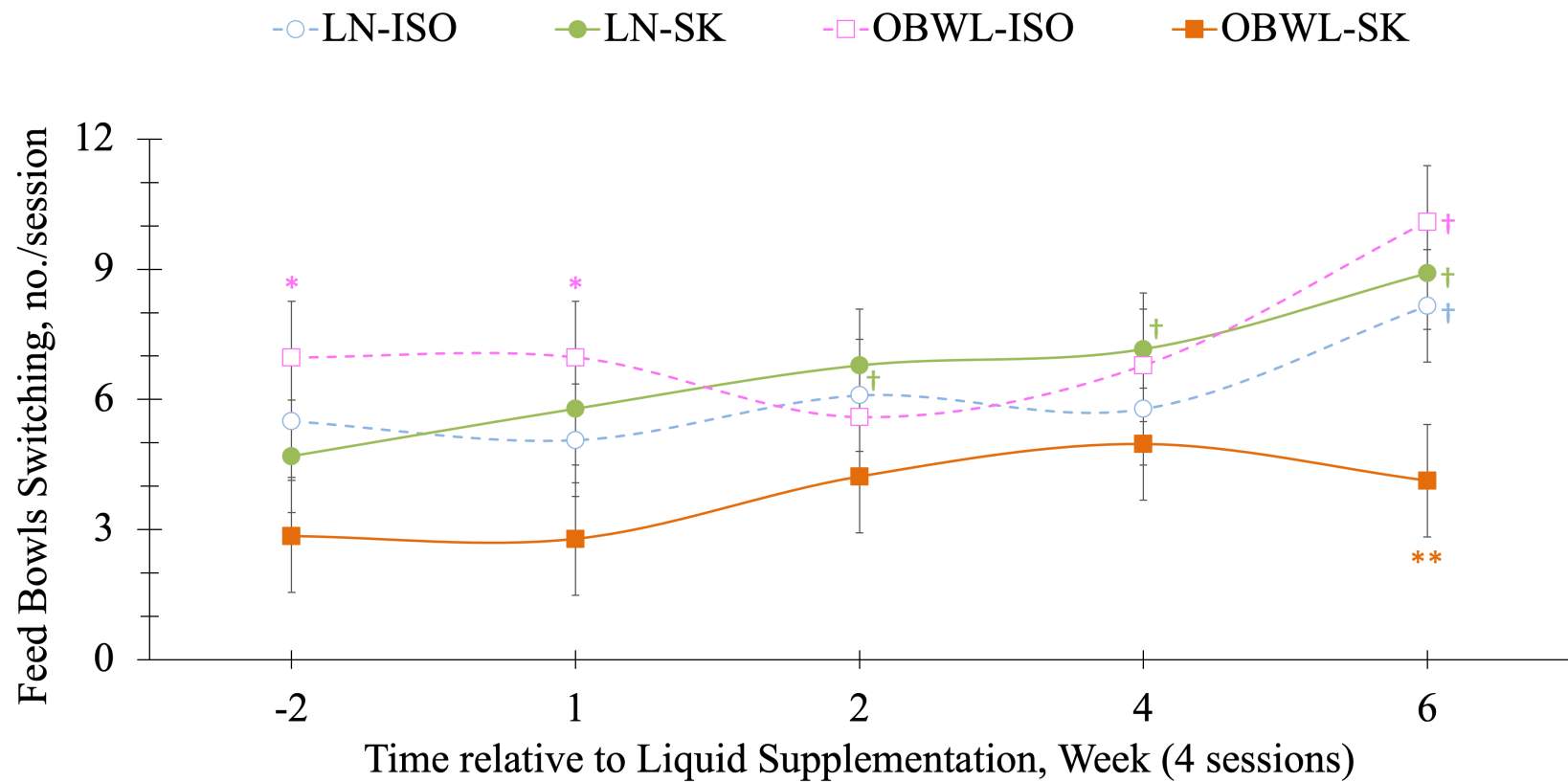


Figure 3.8 Drink duration (% of observations; TRT*Week, $P = 0.005$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week 1 value. *OBWL-ISO differs from LN-ISO and LN-SK at that time point. **OBWL-ISO differs from OBWL-SK at that time point. ***OBWL-ISO differs from all other treatments at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

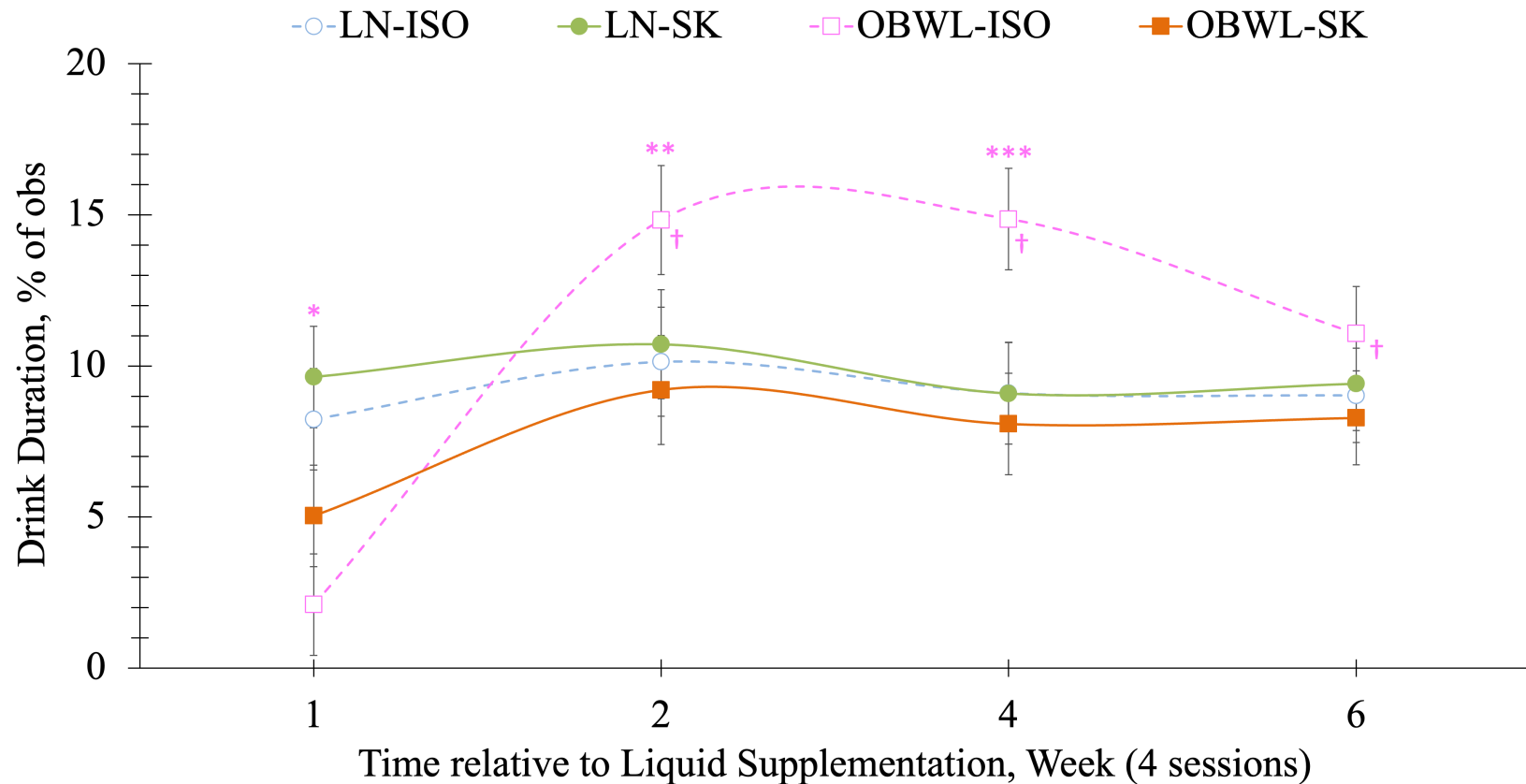


Figure 3.9 Drink frequency (number per session; TRT*Week, $P = 0.014$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week 1 value. *OBWL-ISO differs from all the other treatments at that time point. **OBWL-SK differs from OBWL-ISO and LN-ISO at that time point. ***OBWL-SK differs from all the other treatments at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

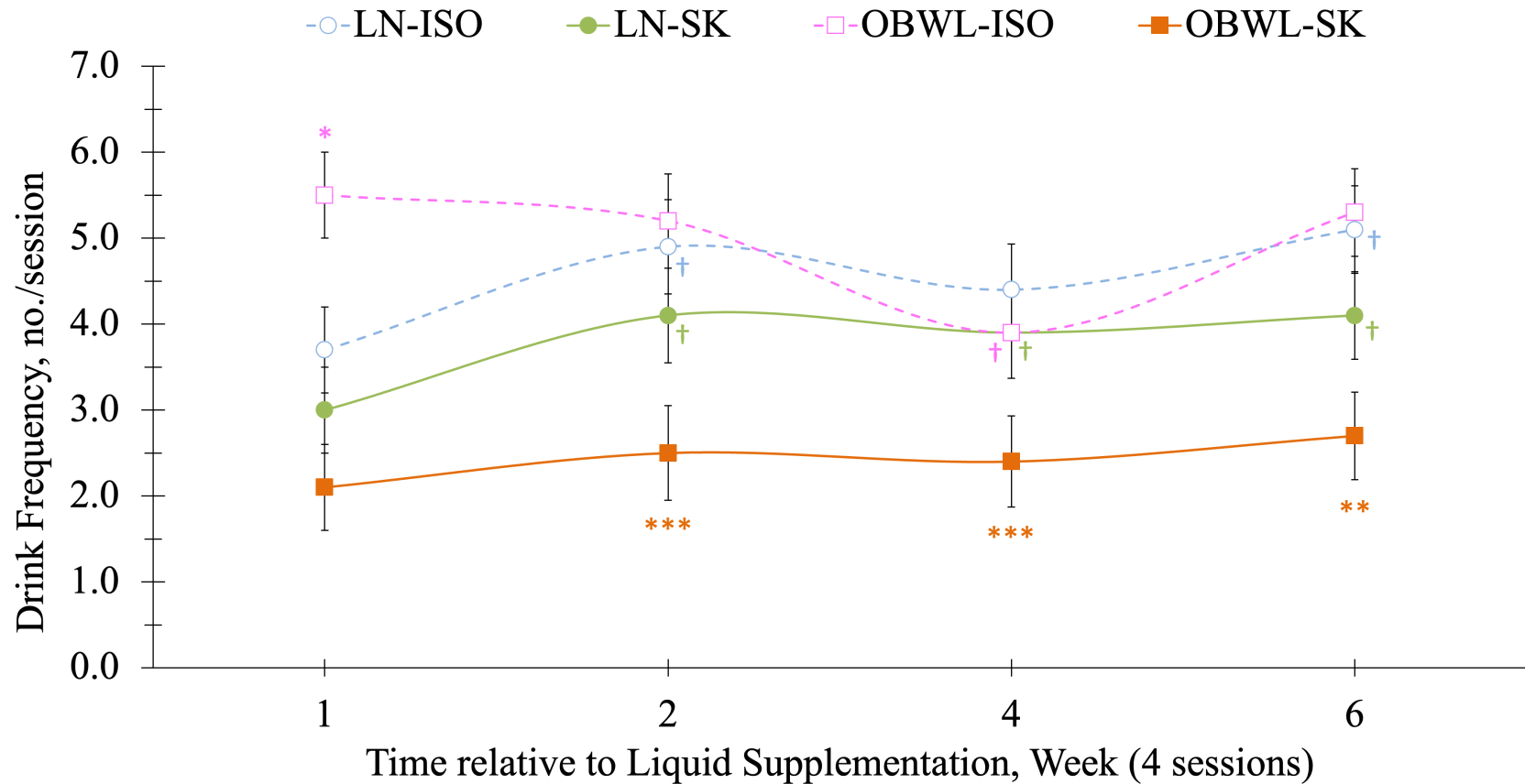


Figure 3.10 Latency to drink (percentage of observations; TRT*Week, $P = 0.059$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week 1 value. *OBWL-SK differs from LN-ISO at that time point. **OBWL-SK had the lowest value at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

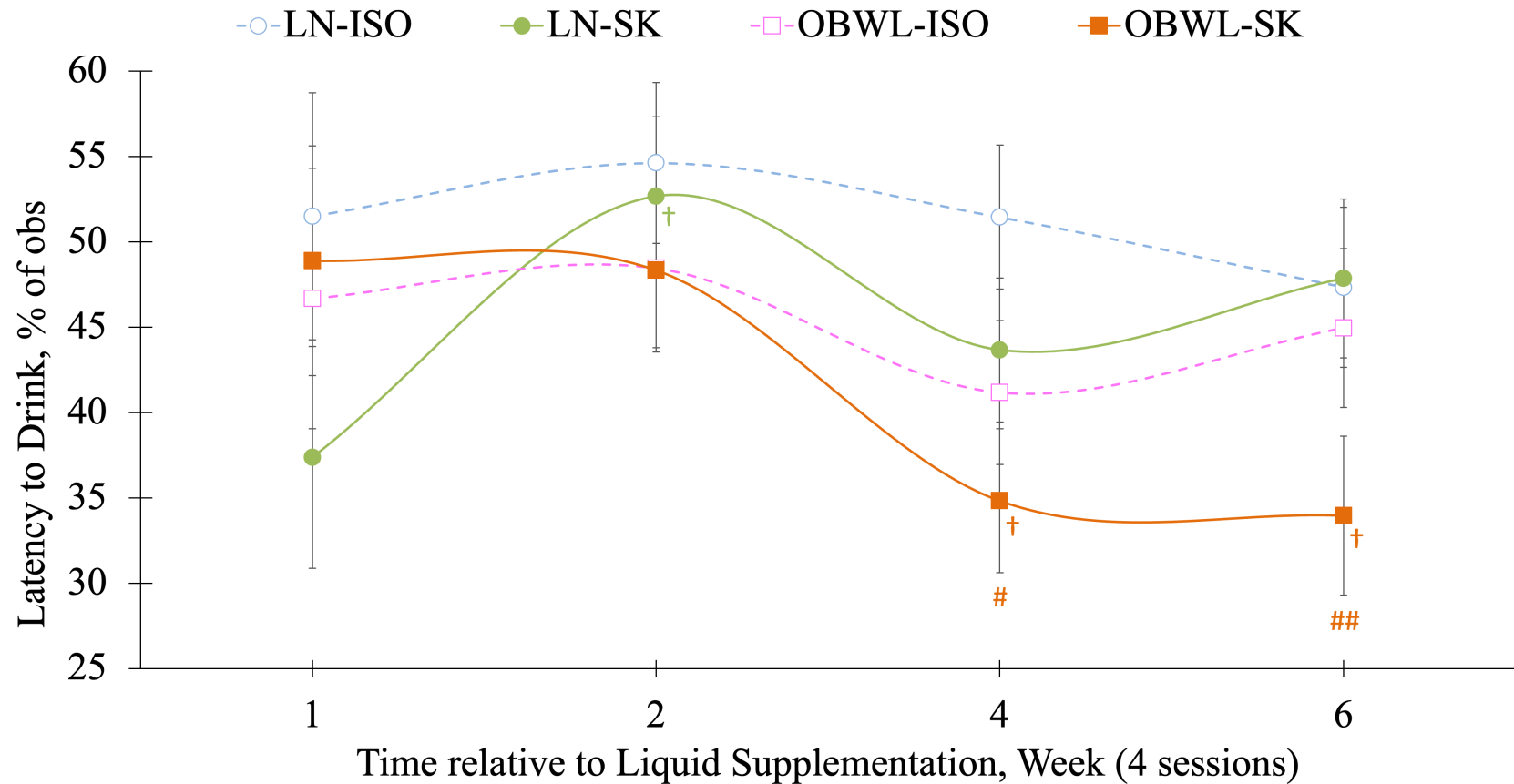


Figure 3.11 Latency to correct paddle (percentage of observations; TRT*Week, $P = 0.016$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *OBWL-SK and OBWL-ISO differ from LN-ISO and LN-SK. **LN-ISO and OBWL-SK differ from LN-SK. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

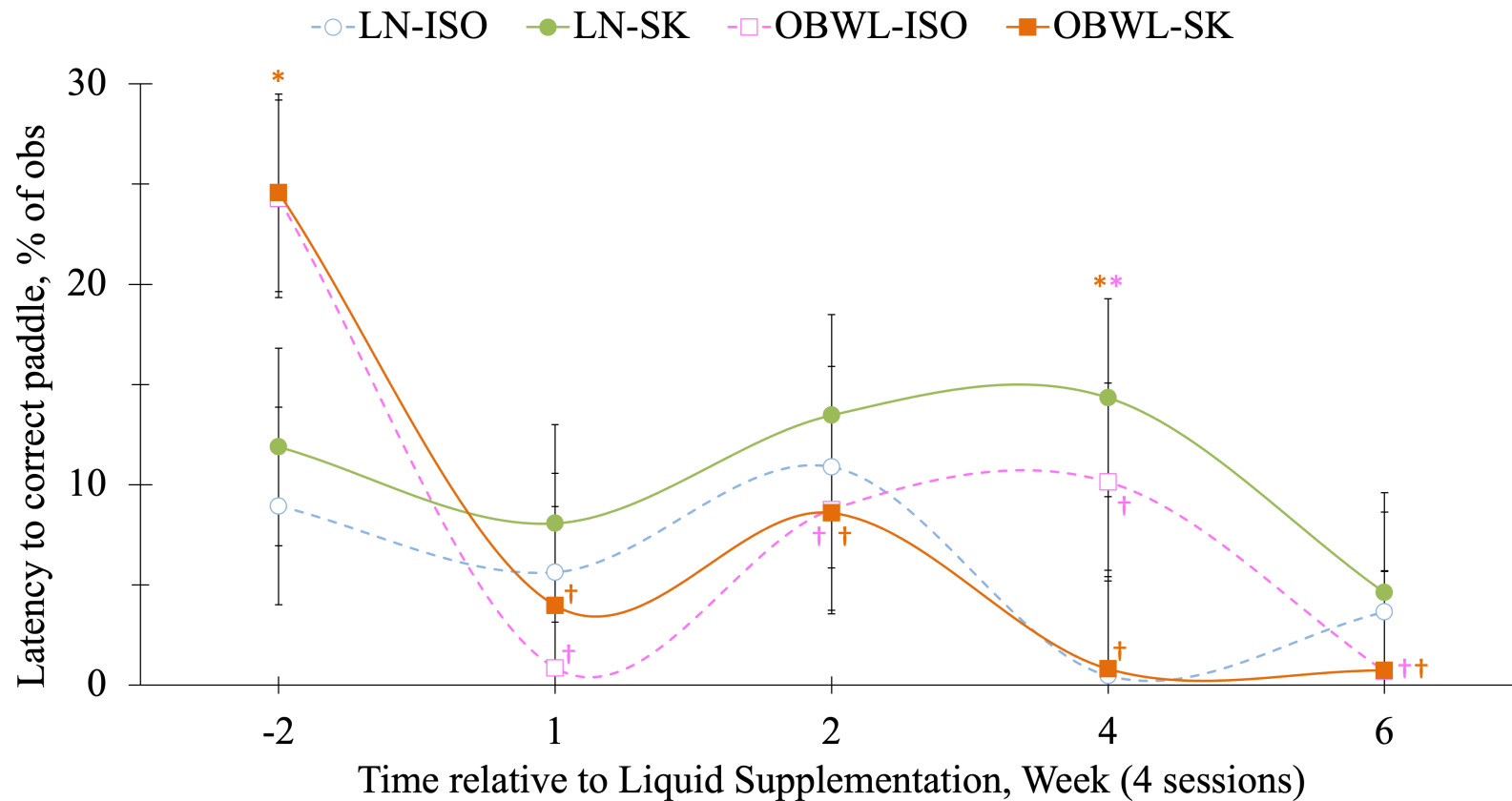


Figure 3.12 Agonistic Frequency (number per session; TRT*Week, $P < 0.001$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *LN-SK differs from OBWL-SK at that time point. **LN-SK differs from all the other treatments at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

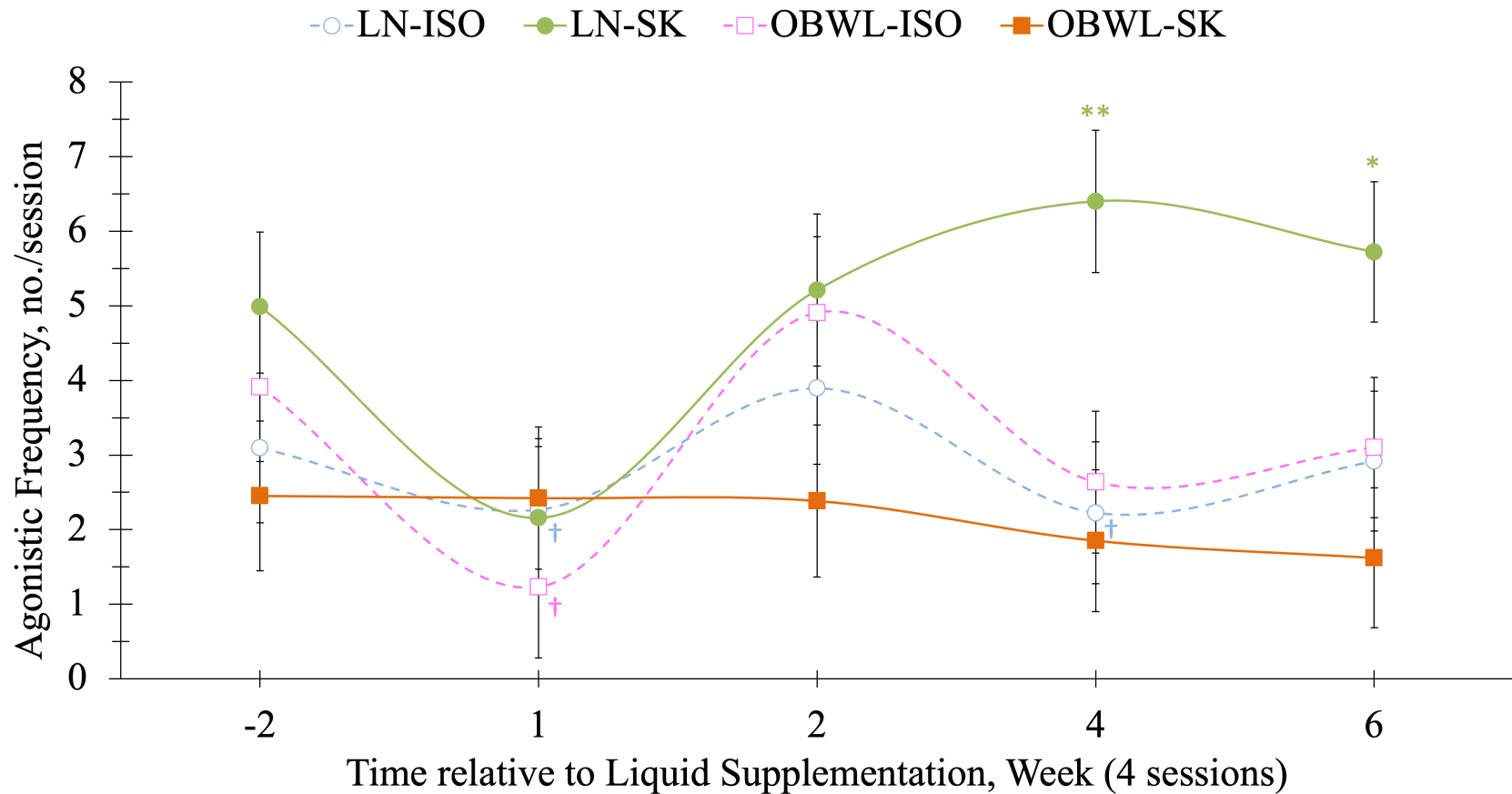


Figure 3.13 Agonistic duration (% of observations; TRT*Week, $P < 0.001$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *LN-SK differs from OBWL-SK at that time point. **LN-SK had the greatest value at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

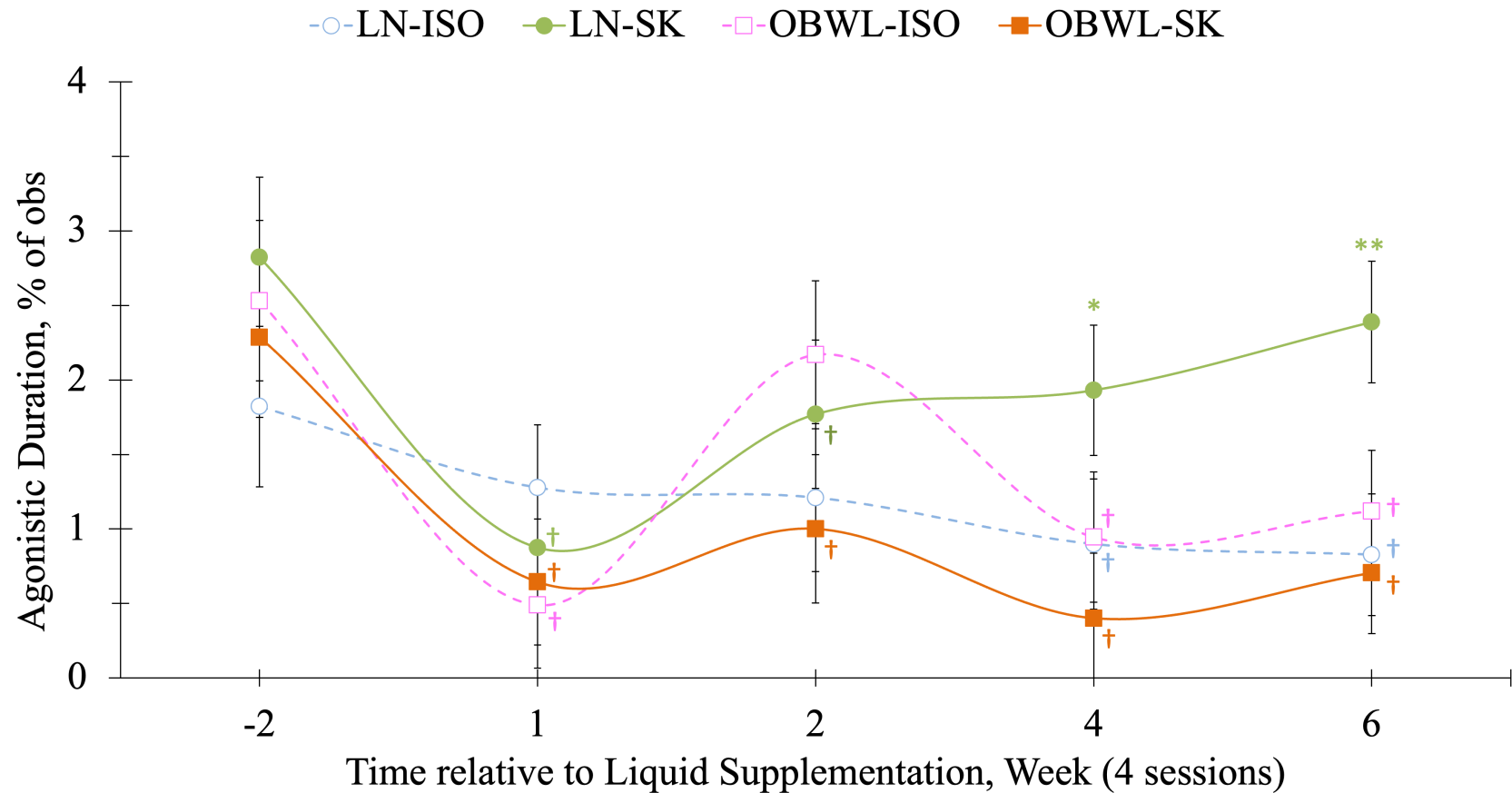


Figure 3.14 Agonistic latency (percentage of observations; TRT*Week, $P = 0.004$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *LN-SK differs from OBWL-SK at that time point. **LN-ISO differs from LN-SK and OBWL-ISO at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

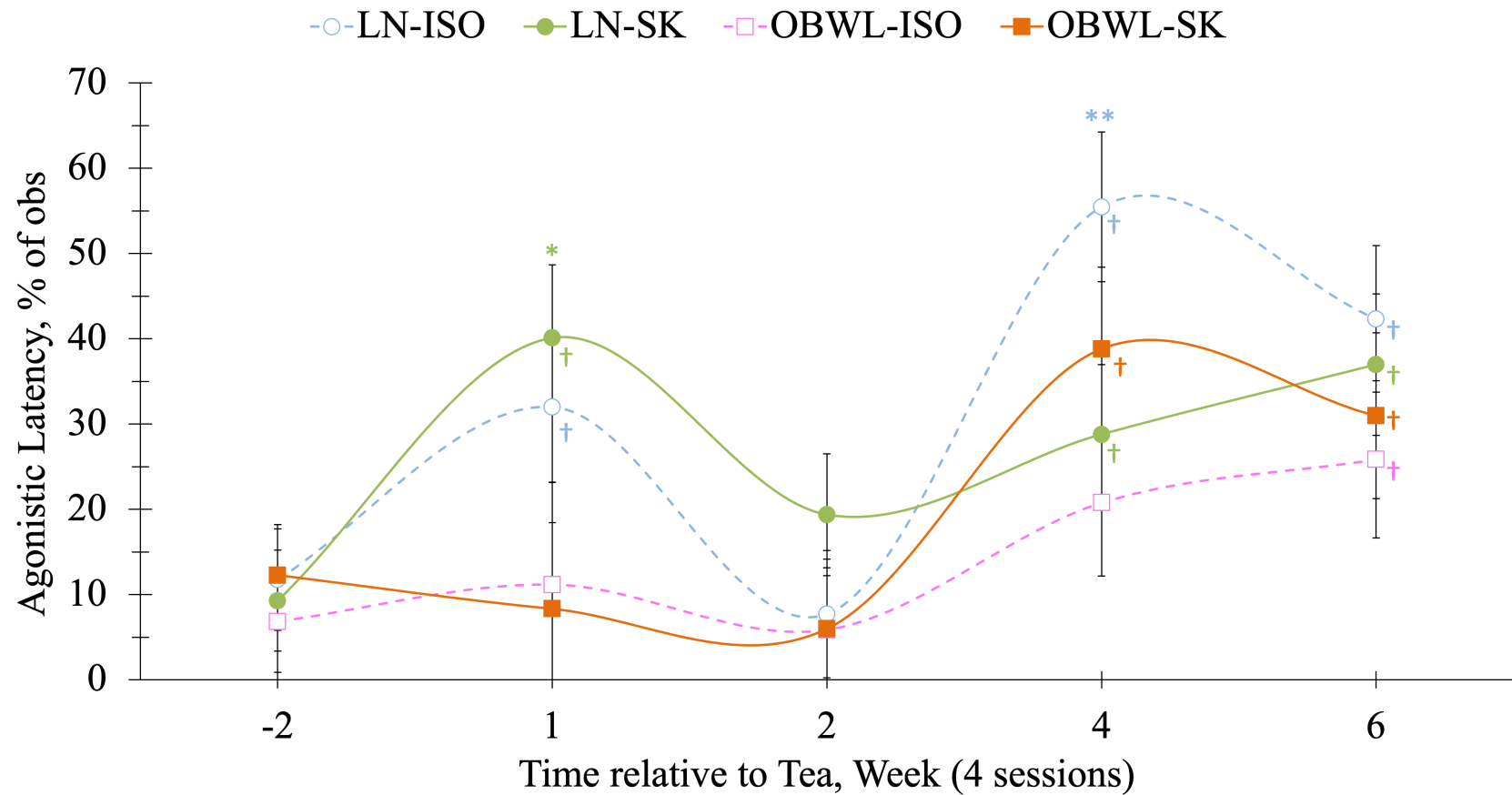


Figure 3.15 Retreat latency (percentage of observations; TRT*Week, $P = 0.003$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week) in individual pigs ($n = 32$). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *OBWL-SK differs from LN-SK and LN-ISO at that time point. **LN-ISO differs from all the other treatments at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

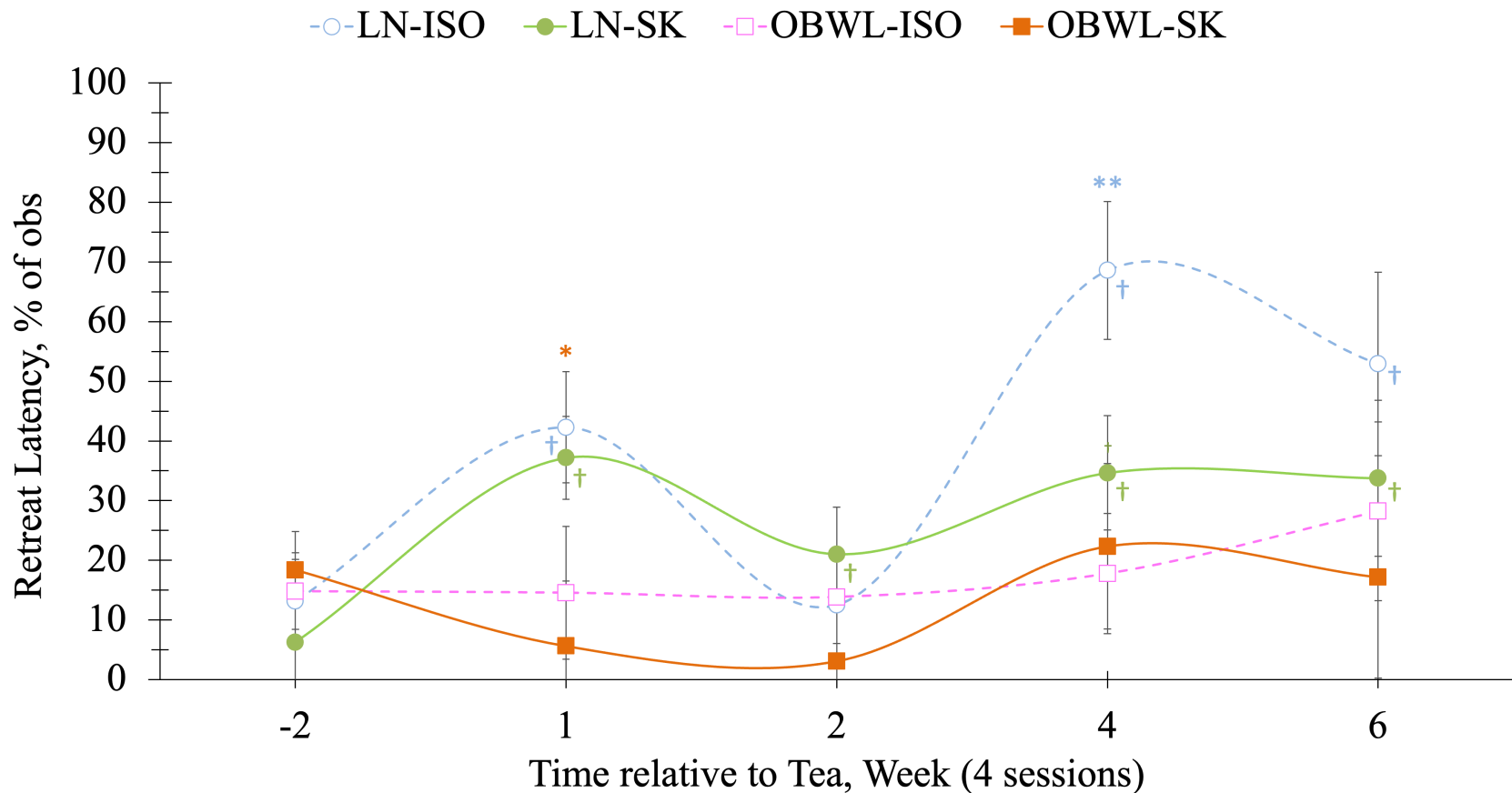


Figure 3.16 Pen (n=16) dominance index (%; TRT*Week, $P = 0.001$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *OBWL-ISO differs from LN-ISO and LN-SK at that time point. **LN-SK differs from OBWL-SK at that time point. ***LN-SK differs from OBWL-ISO at that time point. ****OBWL-SK differs from LN-SK and LN-ISO at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

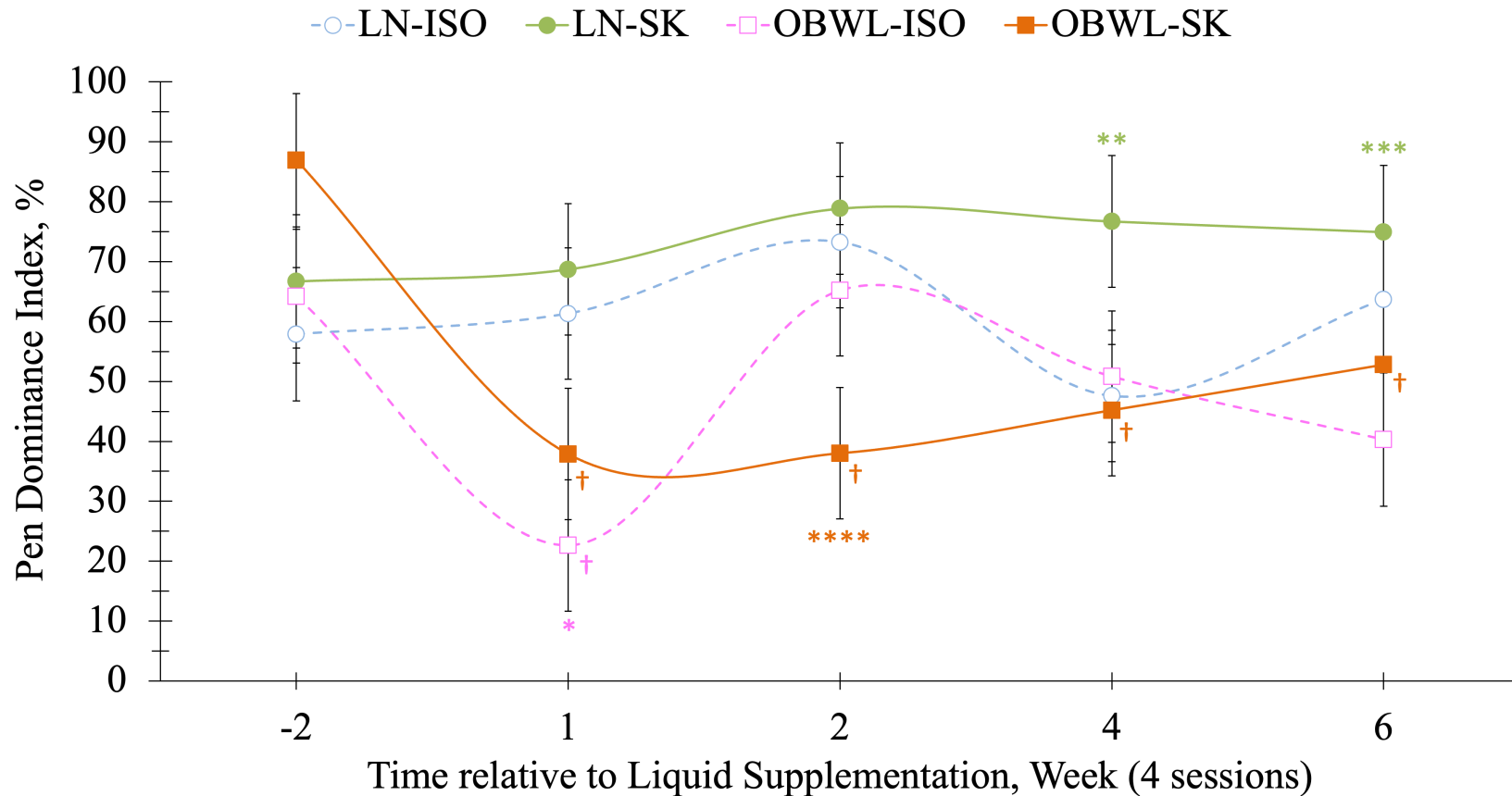


Figure 3.17 Individual (n=32) dominance index (%; TRT*Week, $P = 0.048$) over time relative to liquid supplementation in lean (LN) and obese pigs undergoing a weight-loss program (OBWL), supplemented with either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK). Behaviors were assessed weekly (4 sessions per week). Error bars represent standard error mean. †LS-mean within treatment differs significantly ($P \leq 0.05$; Tukey-Kramer adjusted) from its week -2 value. *LN-SK differs from OBWL-SK at that time point. **OBWL-SK differs from OBWL-ISO and LN-SK at that time point. ***LN-SK differs from OBWL-SK and OBWL-ISO at that time point. LS Means differ ($P \leq 0.05$; Tukey Kramer adjusted).

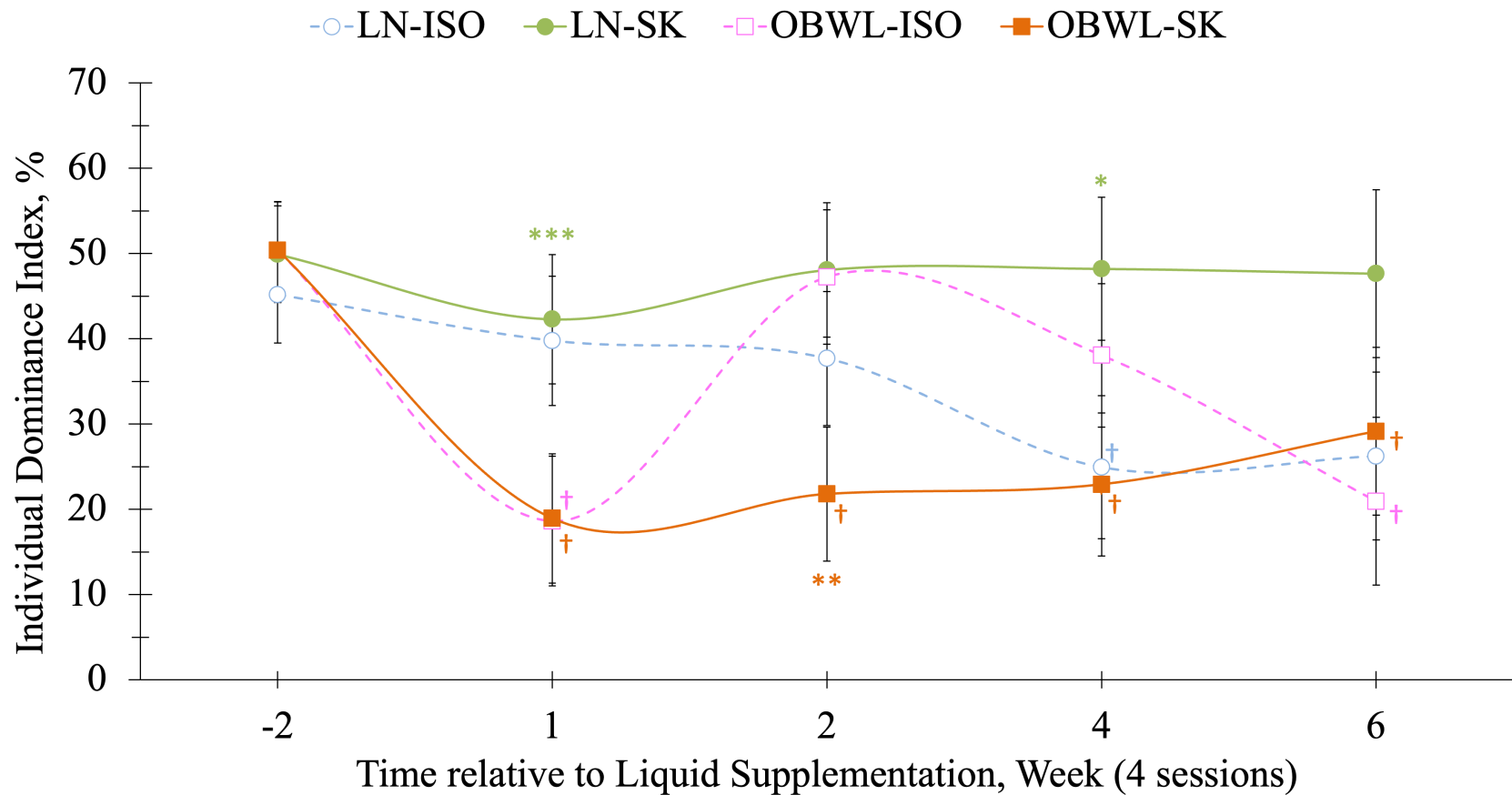


Figure 3.18 Categorical assessment of lean (LN) and obese pigs on a weight loss (OBWL) receiving either an isocaloric control solution (ISO) or fermented sorghum (SK) in relation of motivation to press both correct paddles before and after liquid supplementation. Chi-square analysis $X^2(3, N=1206) = 14.84, P = 0.002$. Values in parentheses represent the expected frequencies from the chi-square analysis (assuming equal distribution), and values in brackets indicate the residual errors.

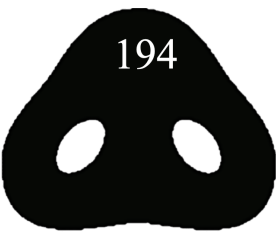
	LN-ISO	LN-SK	OBWL-ISO	OBWL-SK
Before	 199 (186.8) [0.80]	 194 (207.8) [0.92]	 207 (224.3) [1.31]	 219 (200.3) [1.74]
After	 76 (88.3) [1.70]	 112 (98.2) [1.94]	 123 (105.9) [2.76]	 76 (94.7) [3.68]

Figure 3.19 Categorical assessment of lean (LN) and obese pigs on a weight loss (OBWL) receiving either an isocaloric control solution (ISO) or fermented sorghum (SK) in relation to pressing no correct paddle before and after liquid supplementation. Chi-square analysis $X^2 (3, N=599) = 1.1318, P < 0.001$. Values in parentheses represent the expected frequencies from the chi-square analysis (assuming equal distribution), and values in brackets indicate the residual errors.

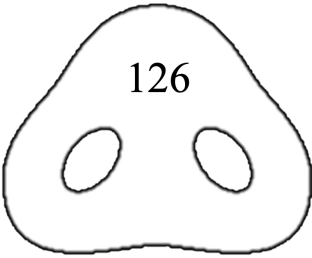
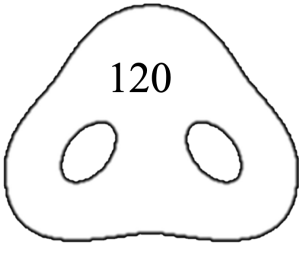
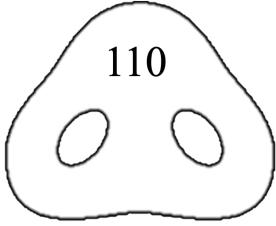
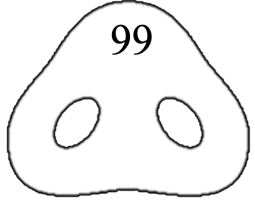
	LN-ISO	LN-SK	OBWL-ISO	OBWL-SK
Before	 126 (129.9) [0.12]	 120 (106.3) [1.75]	 110 (111.7)[0.02]	 99 (107.1)[0.61]
After	 45 (41.1) [0.37]	 20 (33.7) [5.54]	 37 (35.3) [0.08]	 42 (33.9) [1.94]

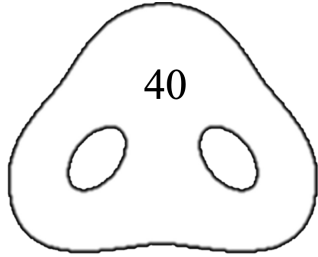
Figure 3.20 Categorical assessment of pigs receiving either an isocaloric control solution (ISO) or fermented sorghum (SK) in relation to pressing both correct paddles before and after liquid supplementation. Chi-square analysis $X^2(1, N=1036) = 22.57, P < 0.001$. Values in parentheses represent the expected frequencies from the chi-square analysis (assuming equal distribution), and values in brackets indicate the residual errors.

	ISO	SK
Before	 236 (272.5) [4.89]	 413 (376.5) [3.54]
After	 199 (162.5) [8.20]	 188 (224.5) [5.94]

Figure 3.21 Categorical assessment of lean (LN) and obese pigs on a weight loss program (OBWL) receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK), relative to pressing both correct paddles during the cognitive task, separated by social status (submissive vs. dominant). Chi-square analysis $X^2(3, N=387) = 38.0036, P < 0.001$. Values represent the total number of both correct paddle presses. Values in parentheses represent the expected frequencies from the chi-square analysis (assuming equal distribution), and values in brackets indicate the residual errors.

	LN-ISO	LN-SK	OBWL-ISO	OBWL-SK
Submissive	 (51.26) [0.44]	 (75.53) [5.02]	 (82.95)[6.92]	 (51.26)[0.00]
Dominant	 (24.74) [0.91]	 (36.47) [10.39]	 (40.05) [14.33]	 (27.74) [0.00]

Figure 3.22 Categorical assessment of lean (LN) and obese pigs on a weight loss program (OBWL) receiving either an isocaloric control solution (ISO) or fermented sorghum kombucha (SK), relative to pressing no correct paddles during the cognitive task, separated by social status (submissive vs. dominant). Chi-square analysis X^2 (3, N=144) = 8.94, P = 0.030. Values represent the total number of both correct paddle presses. Values in parentheses represent the expected frequencies from the chi-square analysis (assuming equal distribution), and values in brackets indicate the residual errors.

	LN-ISO	LN-SK	OBWL-ISO	OBWL-SK
Dominant	 (33.75) [1.16]	 (15.00) [0.07]	 (27.75)[0.81]	 (31.50)[0.20]
Submissive	 (11.25) [3.47]	 (5.00) [0.20]	 (9.25) [2.44]	 (10.50) [0.60]

Chapter 4 - General Conclusions and Future Directions

Abstract

In order to investigate the effects of obesity on the outcomes of social interaction and dietary motivation in pigs, this chapter summarizes behavioral, cognitive, and the potential of sorghum kombucha in research. Overall, the study assessed the impact of fermented sorghum kombucha on body composition and feeding social interactions during early weight loss in Ossabaw pigs, utilizing a mix of pair housing and a cognitive feeder test. Simultaneously, the application of real-time ultrasound provided a non-invasive method to investigate intramuscular fat (marbling), which was unexplored, and created chances to assess the possible impact of sorghum supplementation on meat quality. While AI-assisted behavioral tracking techniques are still presently underutilized in practical applications, they have the potential to significantly improve the monitoring of personalized behavior. Collectively, these insights advocate for a comprehensive approach to agrimedical research and swine production by correlating body condition, meat quality, cognition, and the role of social interactions. Current findings position sorghum kombucha as a novel drink with much yet to be explored.

Keywords: Sorghum Kombucha; Obesity; Cognition; Social Interaction; Ossabaw Pig; Meat Quality

Introduction

Researchers have mostly looked at the physiological effects of obesity in animal models, especially pigs (Lind et al., 2007; Spurlock and Gabler, 2008). They have not paid much attention to the behavioral processes that lead to those effects. This restricted focus aligns with what is happening in biomedical research, where body weight and inflammatory indicators are often seen as separate outcomes (Volkow et al., 2013; Marks, 2015). In swine research,

remarkably few studies have investigated how behavioral traits, such as decision-making, feeding reward preference, or social competition, may affect or indicate the progression of obesity (Elizabeth Bolhuis et al., 2005; Gieling et al., 2010; O'Malley et al., 2022). Behavioral measurements are usually evaluated within carefully controlled cognitive activities or short assessments, seldom included in comprehensive dietary interventions or real-time feeding contexts (Gautier et al., 2020). This signifies a constraint in our comprehension of the mechanisms behind the development and persistence of obesity within dynamic and socially competitive environments (Camerlink et al., 2016; Oldham et al., 2025).

The present study aims to fill this gap, not by eliminating physiological measures, but by situating them within a more comprehensive behavioral framework. We investigated the effects of fermented sorghum kombucha as a potential regulator of metabolic and motivational processes, focusing on its influence on back fat reduction, eating strategies, social interactions, and behavioral flexibility in both lean and obese pigs. By teaching pigs to interact with automated feeders, placing them in dyads, and tracking both body composition and feeding behavior over time, this study offers a rare opportunity to link behavior with physiology in the context of obesity. In doing so, it reflects a shift from studying obesity as a fixed outcome to understanding it as a dynamic process shaped by social environments, decision-making systems, and potential eat behavior modulation.

Timing of Liquid Supplementation

Fermented sorghum kombucha was incorporated throughout the weight-loss phase of this trial, instead of the obesogenic Western diet period, for both scientific and ethical reasons. A primary reason for this was the apprehension regarding possible gastrointestinal complications, specifically small intestinal bacterial overgrowth (SIBO), that could result from combining a

high-fat, high-fructose corn syrup formulated diet with a probiotic-rich beverage. Small intestinal bacterial overgrowth is a condition characterized by the abnormal presence and proliferation of colonic-type bacteria within the small intestine. The SIBO is associated with symptoms such as bloating, diarrhea, nutrient malabsorption, and systemic inflammation, largely due to compromised gut barrier integrity and bacterial fermentation in a region not designed for such microbial load (Dukowicz et al., 2007; Pimentel et al., 2020). Western diets are known to disrupt gut motility and reduce gastric acid production, both of which are critical protective mechanisms against SIBO (Knez et al., 2024; Roszkowska et al., 2024). In this context, the additional microbial load introduced by fermented beverages like kombucha could exacerbate dysbiosis, further impair gut function, and increase the likelihood of inflammatory responses.

Administering sorghum kombucha during the weight-loss phase established a more regulated physiological environment to examine its potential advantages. Upon transitioning pigs from a Western diet to a calorie-restricted diet, their gut environment likely became more stable and amenable to manipulation. This design decision facilitated the investigation of kombucha's impact on body composition and behavior without exacerbating the gut problems already presented by the obesogenic phase. This strategy is consistent with therapeutic techniques that combine probiotic or prebiotic supplementation with deliberate dietary changes, rather than trying to mitigate the effects of detrimental diets while they persist (Álvarez-Arraño and Martín-Peláez, 2021; Hassan et al., 2024). By refraining from administering fermented sorghum kombucha during the obesogenic period, the study also circumvented the propagation of a fallacious narrative suggesting that probiotic supplementation can negate the adverse consequences of a Western-style diet. The study positioned kombucha as a potentially beneficial

aid during metabolic recovery, a period characterized by alterations in body composition, energy utilization, and gut flora reorganization.

Social Dynamics and Group Feeding Systems

Understanding the function of social dynamics in feeding environments is crucial as swine production continues to embrace increasingly automated and group-based systems. Whether in growing-finishing pigs, gestating sows, or experimental cohorts, group housing invariably adds a social component of competition for resources. Pigs must manage hierarchy, timing, and spatial awareness in addition to stress when feeding turns into a social and nutritional event (Verdon et al., 2015; Tu et al., 2022; Agha et al., 2025).

Pigs in this study were housed in pairs and trained to use paddle feeders, which required interaction in order for the animals to get feed. These systems do, however, also require behavioral competence, motivation, and cognitive flexibility, qualities that differ from individual to individual and may be impacted by social standing or physical condition. These dynamics have multiple applications in industry settings. First, individual-engagement feeding systems, like paddle-triggered bowls, enrichment-activated dispensers, or electronic sow feeders, may unintentionally disadvantage animals with altered behavioral profiles or lower social rank. Pigs that are obese, submissive, or cognitively less flexible may access feed less frequently or during off-peak hours in a competitive environment, which could interfere with their nutritional intake or lead to stress-related behaviors (Weng et al., 2009; Zhao et al., 2013; Jang and Oh, 2022).

However, if there is no temporal or spatial partitioning in the system, dominant animals might monopolize feeders. Growth rates, feed conversion efficiency, and even welfare outcomes like aggression, displacement, or frustration can all be impacted by these competitive interactions (Meese and Ewbank, 1973; Boumans et al., 2018). In situations where feeding is restricted or

schedule-bound, such as during pregnancy or research trials, striking a balance between task engagement and social inhibition becomes especially crucial. In addition to learning how to turn on feeders, pigs also need to be prepared to wait, switch, or return based on their pen mate's actions. Dominance, past learning, hunger levels, and perhaps the pig's metabolic state (e.g., lean vs. obese) all play a role in these micro-decisions, such as when to eat and when to retreat. Even when feed is technically available, a pig may completely avoid the feeder if it believes the expense of arguing for a resource outweighs the benefit, which could result in nutritional deficiencies. This emphasizes the necessity of assessing not only what is fed but also the distribution of feeding access from a production perspective (Nielsen et al., 1995; Samarakone and Gonyou, 2009). Systems that fail to take individual differences in behavior or dominance into consideration run the risk of making feed access inequality worse. The increasing use of automated data collection and AI-assisted tracking creates new opportunities for individual feeding behavior monitoring, possibly identifying animals that are performing poorly or being denied access to vital resources (Young and Lawrence, 1994; Pastell et al., 2016; Matthews et al., 2017; Tu et al., 2022; Tiwari et al., 2025).

The idea that feeding environments are behavioral landscapes impacted by social structure, cognitive demands, and physical health rather than just being static delivery points is supported by this study. These results highlight the significance of creating environments that support behavioral variation for commercial producers, particularly those in charge of large pens or precision feeding systems. Redundancy, choice, and feeder accessibility could reduce conflict and encourage steady growth across social levels. The integration of behavioral data with body composition and social indices may ultimately enable more responsive, welfare-conscious, and effective herd management as production systems shift toward precision livestock farming.

Artificial Intelligence Tracking, Feeding and Social Behaviors

Despite tremendous progress, the ability to consistently and objectively observe individual animals, particularly in dynamic group settings, remains a persistent challenge in animal behavior research. Scalable behavior quantification techniques are necessary, exactly the point at which video analysis based on artificial intelligence (AI) can be a game-changer. Scott (2025) and Naiel et al. (2025) effectively used AI to detect zone transitions, latency metrics, and object interactions in maze-based cognitive tests on this same project. These findings laid the foundation for applications in increasingly complex environments by demonstrating AI's ability to consistently extract subtle, task-related behaviors. These maze tasks were perfect for early AI implementation because they were well-structured and had comparatively low spatial demands. However, feeding environments combine social negotiation and cognitive engagement to create a more complex behavioral landscape in development within this study to tracking through AI on this study.

Numerous recent studies demonstrate how AI can be used to monitor pigs in a variety of settings. A YOLOv5-based multi-object tracking model was created by Reza et al. (2025) that could accurately detect both movement and postural changes while following individual pigs in group environments. The ability of depth-camera systems to recognize feeding, standing, lying, and moving behaviors was also shown by Matthews et al. (2017), providing a basis for the early identification of welfare issues. Social network analysis (SNA; Oldham et al. (2024) and artificial intelligence Agha et al. (2025) were combined to produce centrality metrics that revealed group cohesion levels and individual social roles. By employing vision transformers to identify subtle behaviors like head-to-rear contact, an interaction that human observers frequently overlook. Researchers would be able to review all of the videotaped sessions if AI

tools were applied to the feeding tests in this study, creating complete datasets from the animals across timepoints and treatments. This would facilitate more in-depth examination of decision-making patterns (such as switching bowls or coming after a partner), persistence at the feeder, and hesitation or avoidance, all of which may be markers of social standing and cognitive adaptability. Such behavioral data could become effective predictors of learning outcomes when paired with metadata on treatment, obesity status, or performance outcomes.

Non-task behavior is also susceptible. Pigs can be tracked throughout their daily routines using AI-based pen tracking, which records the amount of time they spend exploring, lying alone, interacting with a partner, and participating in enrichment activities (Kashiha et al., 2013; Matthews et al., 2016; Melfsen et al., 2023). A more thorough understanding of baseline temperament, activity level, and social affiliation patterns is made possible by these metrics. These elements are essential for comprehensive behavioral profiling. Moreover, continuous data collection facilitates the early detection of emerging, nuanced patterns, such as indicators of social exclusion, diminished motivation, or even physical discomfort, prior to their manifestation. This allows for more personalized assessments and promotes welfare interventions in large-scale systems that are more responsive (Matthews et al., 2016; Berckmans, 2017).

This move to AI is also important because it makes behavior research more accessible by getting rid of observer bias, making results more consistent, and freeing researchers from time-consuming annotation processes. Automated behavior monitoring is becoming not only possible but also necessary in swine research and production settings as models become more accurate and accessible, the next step in this study.

Production Impacts: Rethinking Obesity in Swine Systems

Obesity in swine production is usually disregarded or even encouraged. However, a thorough examination of reproductive outcomes shows that there are serious repercussions associated with high adiposity. Although sow herd productivity is largely dependent on reproductive efficiency, the prevalent industrial viewpoint that "fat is good" ignores the known disadvantages that overweight sows may experience prior to, during, and following pregnancy. In addition to reduced colostrum production and increased oxidative stress in the placenta, a systematic review by Muro et al. (2023) shows that sows with body condition scores above the optimal range (3.0–3.5 on a 5-point scale) have higher stillborn rates, longer farrowing durations, and increased perinatal mortality. On the other hand, compared to sows kept within an optimal range, both overconditioned and underconditioned sows displayed lower lifetime productivity.

This was corroborated by a large-scale field study (involving more than 1500 sows) that showed a curvilinear relationship between sow backfat and reproductive performance: sows with a body condition score of approximately 3.2–3.6 had the highest number of live piglets and the shortest weaning-to-conception intervals, while sows with backfat exceeding ~21 mm experienced a higher rate of stillbirths, a slower return to estrus, and a higher incidence of postpartum dysgalactia syndrome (Cheng et al., 2019). The next generation is also impacted by obesity. According to a study on litter variability, sows with thicker backfat had litters with higher within-litter birth weight variation, which can have a detrimental effect on the survival and homogeneity of piglets. While variation raises the risk of mortality and necessitates more work, uniform birth weights are essential for efficient post-weaning management (Muro et al., 2023).

In addition, boars fed high-energy diets showed changes in libido, delayed ejaculation, and mounting. These effects imply that energy intake has an equal impact on male and female reproductive performance (Wang et al., 2016). All things considered, an increasing amount of scientific data indicates that obesity is a serious risk to herd reproductive performance that goes beyond finishing metrics. Increased mortality, smaller litters, longer breeding intervals, and worse libido are all quantifiable disadvantages for overweight sows and boars that lower productivity and profitability in a commercial setting. Given this, swine obesity merits much more attention.

This problem is made worse by the housing systems in place today. Sows and gilts are kept in restricted areas like gestation and farrowing crates in a lot of commercial operations. However, animals' capacity to stand, lie, and change positions is hampered when they become obese or overconditioned. This mechanical restraint raises the possibility of pressure injuries, such as skin lesions, leg abrasions, and shoulder sores (Bonde et al., 2004). In addition to being unpleasant, these injuries endanger nursing success, longevity in the breeding herd, and general welfare results. Therefore, obesity is a spatial and biomechanical welfare issue as well as a metabolic and reproductive one. Particularly in confined systems, animals in less-than-ideal physical condition are more likely to suffer during standard housing and husbandry procedures. For sows to be able to nurse piglets effectively and without physical hindrance, they must be able to perform their natural behaviors, such as standing and lying, without experiencing discomfort.

Accordingly, it is essential to maintain ideal body condition throughout the entire production cycle, not only for fertility but also for injury avoidance, crate compatibility, and sow retention. In contemporary swine systems, this viewpoint promotes a more comprehensive

approach to body condition management by viewing it as a characteristic that directly affects housing design, health, and humane treatment rather than just as a numerical backfat target.

Meat Quality Traits

In response to consumer demands for lower-fat meat, the U.S. pork industry made a dramatic shift toward producing leaner carcasses beginning in the early 1990s. By means of rigorous genetic selection, improved feeding techniques, and modified management procedures, producers were able to reduce market hogs' external fat by about 39% (Cassens, 2000). Although this satisfied consumer demand, there was a trade-off: lean pork loses flavor, juiciness, and tenderness, qualities that are crucial for a high-quality meal, by eliminating intramuscular and subcutaneous fat. Pork lacks a common framework for quality, in contrast to the standardized grading systems used in the beef industry (Rosenvold and Andersen, 2003). Breeders and processors, however, now understand that marbling, also known as intramuscular fat (IMF), is essential for sensory appeal. Improvements in intramuscular fat, which has moderate-to-high heritability (0.31–0.62), can improve palatability without appreciably increasing external fat as long as it is incorporated into selection indices (Wood et al., 2008).

This dynamic is emphasized by heritage breeds, such as the Iberian pig, which are prized in Spain for their ability to produce Jamón Ibérico, a type of high-quality, dry-cured ham. The Iberian pigs have genetic characteristics that support high intramuscular fat, characteristic marbling, and elevated levels of oleic acid, which is comparable to the "healthy fats" found in olive oil. The rich flavor profile and mouthwatering texture of Iberian pork are attributed to the increased oleic acid content in intramuscular fat during fattening (Muriel et al., 2004; Daza et al., 2007; Carrapiso et al., 2020). Similar to this, Duroc pigs, which are frequently used as terminal sires, are renowned for their exceptional meat quality, combining flavor, juiciness, and

tenderness in what is sold as "Certified Duroc Pork," an indication that buyers are willing to pay more for reliable, high-quality pork. A crucial lesson is also illustrated by the difference between Duroc genetics and selection for lean gain: aggressive backfat reduction may inadvertently lower intramuscular fat and impair the quality of tender eating (Suzuki et al., 2005; Sirtori et al., 2011). Even as backfat decreases, sensory panels consistently give high-intramuscular pork higher ratings for flavor and juiciness. Thankfully, deliberate selection for marbling can decouple these traits, resulting in lean carcasses with acceptable palatability, because backfat and intramuscular fat have a positive genetic correlation (0.3–0.64; Lo et al., 1992).

The thickness of the back fat and the area of the loin eye were measured in the current study using ultrasound imaging. Recent developments have made it possible to estimate intramuscular non-invasively using ultrasound across the longissimus, allowing for the evaluation of marbling in live animals (Newcom et al., 2002). This is revolutionary for both genetic improvement and production management. Keeping an eye on these characteristics in conjunction with kombucha treatments may reveal minute changes in tissue composition or intramuscular fat deposition. To date, there is no current data regarding sorghum kombucha's possible impact on meat quality. In certain species, polyphenols have been demonstrated to alter antioxidant activity and lipid metabolism, which may have an impact on fat composition and marbling persistence (Zhou et al., 2016; Liu et al., 2021; Xu et al., 2022). However, any conclusions regarding the effect of kombucha on meat quality must remain tentative as this study lacks direct measurements of intramuscular fat or fatty acid profiles.

Limitations

A portion of the feeder sessions were coded in Observer XT, despite the development and validation of an ethogram. This makes it more difficult to evaluate behavioral consistency over

time and to interpret longitudinal behavioral changes. A more thorough understanding of learning patterns, social contest dynamics, and individual feeder strategy variability would probably result from a full video analysis of every session. Similarly, at the time of this thesis, AI-assisted behavior tracking was still in its early stages and had not yet been fully applied to pen-level activity or feeding behavior. The application of AI to more intricate, multi-individual interactions at the feeder and within the pen is still a goal, even though initial validation was carried out during cognitive testing in the maze task. This made it more difficult to track affiliative behaviors, activity budgets, or dominance relationships over time.

Conclusion

From behavioral, physiological, and cognitive assessment perspectives to determine the impact of fermented sorghum kombucha on Ossabaw pigs undergoing a weight loss program, obese pigs exhibited modified social behavior and eating desire, particularly under cognitive stress. Obese pigs supplemented with polyphenol-rich kombucha exhibited greater back fat percentage change and average daily gain, besides, it exhibited enhanced eating engagement, indicating possible advantages for motivation and behavior. Ultrasound evaluations facilitated alterations in body composition and present a promising instrument for investigating intramuscular fat and its possible effects on meat quality. Although the first results are promising, more research is necessary, especially given the complexity of obesity, the unresolved questions surrounding the obesity paradox, and the need to further specify the effects of fermented sorghum kombucha.

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Appendix A - Nutritional composition of experimental diets (MetS, 5L80 LabDiet and DuMor) used in Ossabaw pig feeding trials

		MetS	5L80 Lab Diet	DuMor
Nutrients	Protein, %	16.9	16.2	15
	Arginine, %	0.92	0.97	.
	Cystine, %	.	0.28	.
	Glycine, %	.	0.73	.
	Histidine, %	0.47	0.44	.
	Isoleucine, %	0.95	0.69	.
	Leucine, %	1.44	1.37	.
	Lysine, %	1.16	0.88	0.85
	Methionine, %	0.36	0.35	.
	Phenylalanine, %	0.86	0.77	.
	Tyrosine, %	0.71	0.48	.
	Threonine, %	0.67	0.6	.
	Tryptophan, %	0.22	0.19	.
	Valine, %	1.09	0.76	.
	Alanine, %	.	0.99	.
	Aspartic Acid, %	1.54	1.79	.
	Glutamic Acid, %	3.04	3.65	.
	Proline, %	1.09	1.22	.
	Serine, %	0.81	0.83	.
	Taurine, %	0	0	.
	Fat (ether extract), %	19.8	3.6	.
	Fat (acid hydrolysis), %	19.7	4.7	.
	Crude Fat, %	.	.	3
	Cholesterol, ppm	20,000	0	.
	Linoleic Acid, %	1.45	1.86	.
	Linolenic Acid, %	0.13	0.18	.
	Arachidonic Acid, %	0	0	.
	Omega-3 Fatty Acids, %	0.13	0.2	.
	Total Saturated Fatty Acids, %	10.69	0.63	.
	Total Monounsaturated Fatty Acids, %	4.98	0.73	.
	Polyunsaturated Fatty Acids, %	1.33	.	.
	Fiber (Crude), %	.	5.6	5
	Fiber (Max), %	5.1	.	.
	Neutral Detergent Fiber, %	11.9	17.4	.
	Acid Detergent Fiber, %	6.2	6.9	.
	Nitrogen Free Extract (by difference), %	42.3	57.8	.
	Starch, %	28.35	37.3	.
	Sucrose, %	1.67	1.81	.
	Fructose, %	18.64	.	.
	Glucose, %	2.29	.	.
	Lactose, %	0.01	.	.
	Total Digestible Nutrients, %	51.8	75.9	.

		MetS	5L80 Lab Diet	DuMor
Energy	Gross Energy, kcal/gm	4.15	3.83	.
	Calories by Protein, %	16.3	.	.
	Calories by Fat (ether extract), %	42.9	.	.
	Calories by Carbohydrates, %	40.8	.	.
Minerals	Ash, %	5.9	6.6	9
	Calcium, %	1.03	1	1
	Phosphorus, %	0.81	0.83	0.65
	Phosphorus (non-phytate), %	0.65	0.63	.
	Potassium, %	0.83	0.96	.
	Magnesium, %	0.21	0.19	.
	Sulfur, %	0.13	0.22	.
	Sodium, %	0.25	0.21	0.8
	Chloride, %	0.38	0.41	.
	Fluorine, ppm	38.7	42	.
	Iron, ppm	288	470	.
	Zinc, ppm	222	220	80
	Manganese, ppm	40	97	.
	Copper, ppm	6	30	.
	Cobalt, ppm	0.3	0.53	.
	Iodine, ppm	1.14	1.1	.
	Chromium (added), ppm	2.17	0.01	.
	Selenium, ppm	0.36	0.58	0.3
Vitamins	Carotene, ppm	0.1	3	.
	Vitamin K, ppm	2.9	2.6	.
	Thiamin, ppm	10	9.2	.
	Riboflavin, ppm	13.9	14	.
	Niacin, ppm	64	77	.
	Pantothenic Acid, ppm	40	40	.
	Choline, ppm	659	950	.
	Folic Acid, ppm	4	4	.
	Pryidoxine, ppm	6.41	6	.
	Biotin, ppm	0.4	0.4	.
	B12,mcg/kg	78	78	.
	Vitamin A, IU/gm	6	6	.
	Vitamin D3 (added), IU/gm	1.1	1.1	.
	Vitamin E, IU/kg	110	110	.
	Ascorbic Acid, mg/gm	.	0	.
	Asorbic Acid, ppm	0	.	.

Appendix B - Plant view layout of the Large Animal Research Center (LARC) facility with the housing arrangement, including the location of individual pens (1-8), PLC, and the maze.

