Foundational sorghum kombucha research in swine: preference, cognition, and social testing

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Abstract

The use of swine has the potential to extend greatly beyond their current facets of utilization. The combined advancements in the methodology of behavioral collection and precision animal technology allow for the refinement of swine testing procedures regarding both efficiency and welfare. This thesis will introduce the development of procedures using a novel 3-pod maze to assess preference as well as the social impacts of an in-pen cognitive feeding test.

Study 1 was conducted to measure pig preference for three different strains of fermented sorghum tea utilizing a three-pod radial maze. The first phase served to eliminate neophobia by acclimating in pairs with each bowl containing a control isocaloric solution of sugar water (positive control). The second phase conditioned the pigs individually using the positive control in one arm and distilled water (negative control) in the other two arms. The first experimental phase consisted of testing of testing the preference of three different versions of fermented sorghum tea. Each solution utilized a different strain of commercial sorghum: 1) Raw white, mild flavor profile, 2) Raw sumac, medium flavor profile, and 3) Roasted sumac, bold flavor profile. There was no clear preference between strain so in the second experimental phase the three teas were mixed and put into one pod on a rotation with the positive and negative control. Results showed that the 3Tea was the most desirable treatment as it had the highest consumption volume over the positive and negative controls. Total duration spent in the pod as well as duration of head over bowl behaviors was greatest for pods containing the 3Tea mixture and lowest for the negative control. In conclusion, the pigs preferred the 3Tea mixture over a sweetened water. This study provides evidence that pigs do not have a strong aversion to novel, sour, and bitter flavors as previously thought.

Study 2 was conducted to test a prototype for an in-pen cognitive feeding task. Pigs were exposed to a cognition task that required them to push a series of paddles to receive a food reward. After a conditioning phase the pigs were randomly assigned a pattern for individual acquisition. The pattern consisted of either alternating, inner or outer paddles. Once the pigs completed the individual acquisition phase they were exposed to the test with the same pattern in pairs. Performance on the task was assessed through latency, duration, and frequency measures. The paired phases allowed for the analysis of social hierarchy and cooperation on task performance with an end goal of assessing if sociality and format impacted test performance. Social behaviors assessed included advance, retreat, and neutral. Frequency data for these social behaviors was utilized to calculate dominance indices and determine social status. Format did not have a significant impact on overall performance. However, the outside format had the highest rate of correct paddle contact leading to the extrapolation that it was the easiest for the pigs to uptake. Submissive pigs also had a significantly higher number of overall paddle presses as well as presses on the correct paddle, indication higher motivation and contrafreeloading status when faced with a cognitive task. Dominant pigs were likened to freeloaders as their higher social status was their main tool for meal acquisition.

The results of these studies are assessed together through hematology results reported in the final chapter. Blood was collected from all pigs pre and post study. Dominant and submissive status assigned in study 2 was used to assess hematological differences between pen-mates and the potential health benefits of the fermented sorghum tea consumption. Dominant pigs showed higher levels of plasma glucose, triglycerides, and cholesterol. Dominant pigs also had higher overall body weight correlating with higher fat and inflammatory markers seen in the blood results. Despite an increase in size dominant pigs experienced a decrease in all of these variables post-study, rendering further investigation of the effects of fermented sorghum essential.

The development of procedures for these studies will assist in the refinement and ultimate advancement of the role swine can play within the scientific community. A refinement of behavioral and cognitive data collection will create for more efficient and ethical housing of swine in production and translational studies. The methodology outlined within this thesis can be used as a guide for future studies assessing swine preference, cognition, and social hierarchy.

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Chapter 1 - Review of Literature

Introduction

The long-term objective of our research is to develop novel sorghum-based products to address populations affected by obesity and food insecurity. These issues have become very prevalent in modern society and presents the paradox that members of lower socioeconomic classes are more likely to experience food insecurity (McLaren et al., 2007; Anekwe et al., 2020; Benusic & Cheskin, 2021). The state of being obese can create many issues including behavioral and neurocognitive dysfunction. The onset of this issues largely stemming from low-grade inflammation that the body experiences due pro-inflammatory cytokine release from hypertrophic adipocytes (Hildebrandt et al., 2023). When this inflammation becomes chronic it can negatively impact the body in many ways, one of which being brain function. As a result, long-term behavioral changes can be indicative of neurodegenerative conditions such as dementia and Alzheimer's disease (Rao et al., 2012; Singhal et al., 2015).

Before we could address the long-term objective, we needed to first determine: 1) if adult Ossabaw pigs would possess an aversion to a potentially sour/bitter beverage made out of fermented sorghum. 2) Develop and refine the methodology for a novel in-pen cognitive test that can measure cognitive abilities in group housed swine. 3) Establish baseline hematology parameters for a healthy group of adult Ossabaw swine to act a control to future studies utilizing an obese vs. lean model. A byproduct of the development of these objectives are the improvement to the behavioral data collection for animals used for research and consumption. The use of behavior and cognition as indicators for affective state and overall welfare is a developing practice (Candiani et al., 2008; Roelofs et al., 2016; Miller et al., 2020). There is a great deal to be experimentally learned from the testing of animal cognition and behavior. The cognitive capabilities of swine have the potential to assess the impacts of pharmaceuticals and other experimental conditions more effectively than a rodent model (Singh et al., 2016; Henze et al., 2019). The cognitive state of an animal also has the potential to be a stronger preliminary indicator for certain pathologies and an overall decreased welfare state (Kirkwood & Hubrecht, 2001; Mendl & Paul, 2004). The cognitive performance of an animal is the bridge between the mental welfare state and physiological health. A mastery of behavioral and cognitive collection and interpretation will lead to better experimental practices and understanding of results.

The feeder prototype (by Hulbert and ShapeMaster) provides an in-pen assessment of cognitive flexibility within a social context. The ability for the pigs to solve the feeder and improve over time is a strong assessment of cognitive welfare This novel feeding test can be readily be used in tandem with any other experimental procedures that the subjects are undergoing as it does not require any extra space and minimal labor. Preference testing in swine has been explored (Solà-Oriol et al., 2011; Horback et al., 2016; Fleming & Dilger, 2017; Paggi et al., 2020). However, it has not been commonly executed using a radial maze. The use of the radial maze provides the ability to test preference for a greater number of variables. The radial maze is also a strong indicator of decision making as the pig must return to the center of the maze in between each choice. These factors cannot be achieved with the more commonly used T-maze and thus provides a more robust experimental assessment of preference.

The preference and cognitive feeder tests along with hematological measures were also collected in order to establish baseline blood parameters for a group of healthy Ossabaw swine. These values also allow for the gathering of preliminary information on the potential impact of fermented sorghum products on the pigs. Sorghum is a highly sustainable grain that contains a high concentration of polyphenols (Chipanshi et al., 2003; Xu et al., 2021). These polyphenols carry anti-microbial, anti-inflammatory, antioxidant, and probiotic benefits (Jayabalan et al., 2008; Olawole et al., 2019). When fermented these beneficial characteristics are amplified thus creating the potential for a novel product marketable to nutritionally insecure populations (Song et al., 2019; Olawole et al., 2019; Otunba et al., 2021; Garzón et al., 2023). However, the issue with utilizing adult pigs for this model is that there is potential for rejection of the bitter/sour flavor of kombucha. There was also a need for baseline hematological data to of healthy adult Ossabaw pigs in order to be able to assess their delineation in future studies utilizing a metabolic model. An in-pen cognitive test has also not been developed using adult pigs, however it has the potential to be highly valuable in biomedical pig studies. The development of testing methodology for the preference maze and feeder prototype allowed for valuable preliminary data collection on the viability of a fermented sorghum product. This makes the radial maze and cognitive feeder system valuable commercial products that can also be efficiently replicated using the procedures outlined within this thesis.

Sorghum

Sorghum is one of the most popular cereal grains in the world due to its sustainability and durability (Chipanshi et al., 2003; Chadalavada et al., 2021). Its abundant availability makes it a perfect option for further development into a product that could supplement nutritionally deficient populations around the world. Kansas is the top sorghum producing state in the country, accounting for 55% of the nation's supply. This equates to 169 million bushels of sorghum in 2023 (USDA, 2023). There are many beneficial properties of sorghum that are amplified through the fermentation process. However, even without fermentation, sorghum contains a myriad of beneficial properties. This can mainly be attributed to the polyphenols primarily found within the pericarp and the testa of the plant (Xu et al., 2021). Polyphenols are secondary metabolites that plants use to defend themselves from things like UV radiation and pathogens (Pandey & Rizvi 2009). They can also be responsible for the bitter and astringent flavors of some foods. The past couple of decades of research have shown that they possess powerful health benefits. The polyphenols found within white and sumac strains of sorghum specifically, possess strong anti-inflammatory properties, as they can inhibit pro-inflammatory cytokines such as IL-1β and TNF-α (Birhanu, 2021).

Populations that are food insecure need access to products that will make them feel full for longer periods of time. In a controlled consumer trial, Stefoska-Needham, and others (2016), conducted a panel to compare hunger and satiety scores of biscuits made with white, red, or brown sorghum against a control group of biscuits made with wheat. Subjects consumed 50 grams of one of the four previously stated treatments after a fasting period of twelve hours. Subjective satiety scores were collected at 8 different time points over the course of four hours. All of the subjects that consumed sorghum biscuits reported significantly higher satiety scores in comparison to subjects that consumed the wheat biscuits. Hunger scores were also lower for the sorghum groups over the course of four hours. This research shows that sorghum can be a promising ingredient in developing products to address satiety and weight control. Extended feelings of fullness are associated with a high concentration of resistant starches (RS) as well as slowly digestible starches (SDS; Xu, 2019). Resistant starch aids in glycemic control as most of it is not digested in the small intestine and therefore, not converted to glucose right away. The smaller amount of digestible starch ultimately results in a slower rise in postprandial glucose and longer feelings of satiety (Huang et al., 2022). Slowly digestible starch works much in the same manner as it is digested within 20-120 minutes creating a low glycemic response (Huang et al., 2018). Resistant starches primarily move onto the large intestine where they function as a form of dietary fiber, further increasing feelings of satiety (Schroeder et al., 2013).

Sorghum also possesses strong free-radical scavenging capabilities. This is credited to their high concentrations of bioactive polyphenols such as gallic acid (Kahkeshani et al., 2019; Xu et al., 2021; Frankowski et al., 2022). These antioxidant properties are also able to be retained through processing. When baked 57-78% of antioxidant activity was retained while 70-100% was retained for extruded products (Awika et al., 2003). These numbers suggest the functionality of sorghum and the potential it could have for a successful product. Although sorghum has beneficial nutrients such as polyphenols, there are some drawbacks such as the heating process decreasing protein bioavailability (Duressa et al., 2018; Xu et al., 2021). Researchers speculate that the very polyphenols that provide a benefit for immune regulation also may interact with protein availability. Nonetheless, this may be remedied by fermentation.

Fermentation

The practice of fermentation is said to reach as far back as 8000 years to the fertile crescent of what is now Iraq. It can be simply defined as the anaerobic breakdown of a carbohydrate into either an alcohol or an acid. This is completed as part of the normal metabolic processes of certain microorganisms (Maicas, 2020). Mainly utilized as a means of food preservation, microbial fermentation produces metabolites that can suppress the proliferation of the microflora that cause spoilage (Ross et al., 2002). Modern-day starter cultures (i.e. SCOBY, symbiotic culture of bacteria and yeast) are often used to induce and control the fermentation process in a wide range of products, such as kimchi, yogurt, sourdough bread and kombucha (Nagaoka et al., 2019; Antolak et al., 2021; Kruk et al., 2021; Rolim et al., 2022). These metabolic by-products are the key to the development of many commercial products. An example being the use of *Lactobacillus delbrueckii* subsp. bulgaricus, and *Streptococcus thermophilus* in dairy products. These bacteria specifically give off lactic acid resulting in the coagulation of the milk protein, thus giving yogurt its polarizing texture (Nagaoka et al., 2019). The fermentation of kombucha using SCOBY will also see organic acid by-products such as lactic, malic, and gallic acids (Harrison & Curtin 2021, Yang et al., 2022). These by-products are what gives kombucha its sour flavor and astringent mouthfeel (Bwamu et al., 2022; Xu et al., 2023).

Fermented products in general have been growing in commercial popularity in the U.S. for their reported health benefits. Fermented food and drinks have an increased nutrient availability as well as antioxidant, antimicrobial, and probiotic properties (Svanberg & Lorri 1997; Sreeramulu et al., 2000; Park et al., 2014; Koh et al., 2021). The large number of microorganisms found within fermented products is greatly beneficial to replenishing and maintaining a healthy gut microbiome (Taylor et al., 2020; Leeuwendall et al., 2022). Recent research on bidirectional communication between the gut and the brain coined, the Gut-Brain Axis, have highlighted the impact that a healthy gut biome can have on neurological function (Mayer et al., 2015; Wang & Wang, 2016; Appleton, 2018; Morais et al., 2021).

The gut-brain axis is a complicated amalgamation of neural, endocrine, immune, and humoral processes. Ultimately it connects the cognitive portion of the brain to peripheral intestinal functioning (Carabotti et al., 2015). The gut can signal to the brain to impact processes such as production and expression of neurotransmitters, mucosal immune regulation and protection of the intestinal barrier. The brain can signal to the gut to alter mucus and biofilm production, motility, intestinal permeability and overall immune function (Carabotti et al., 2015). Many mood disorders possess a comorbidity with gastric upset, while GI diseases like IBS and

IBD are often found to have psychological comorbidities such as anxiety and depression (Cryan & Dinan, 2012; Morais et al., 2015). These pathologies all follow alterations within the gut microbiome. Probiotic treatment for GI diseases like IBS is inconclusive and largely strain specific, making a consistent treatment hard to pin down (Korpela & Niittynen 2012; Barbara et al., 2018; Horvat et al., 2021). This leaves an opening for the increased focus on the impact fermented products could have on these issues. Due to the heightened nutritional bioavailability of fermented products could likely have a more significant impact on the gut and body overall in comparison to just probiotic supplementation alone (Choi et al., 2011).

The natural process of fermentation within the gut has a large impact on overall health (Arora & Sharma, 2011; Liu et al., 2020). It is currently being explored that fermented beverages have the potential to aid in weight management and diabetes prevention (Koh et al., 2021). Diversification of the microbiome through the introduction of fermented products can optimize digestibility and utilization of nutrients. Regular consumers of kimchi have a higher concentration of Actinobacteria, whose presence is negatively correlated with weight gain (Han et al., 2015).

Bryant et al., (2023) postulated that a major trigger for hominid brain expansion was the accidental consumption of novel fermented nutrients. This expansion is thought to have occurred around 800,000 years ago with hominids only developing cooking techniques ~780,000 years ago once the hominid brain had already undergone a period of expansion that can be attributed to the more primitive avenue of accidental consumption of food that had undergone spontaneous fermentation. The adaptive benefits of fermented foods are vast as the conversion of various macromolecules into their more easily digestible counterparts make fermented foods ideal for food in secure populations. The previously discussed benefits of fermented foods created a situation where early humans were becoming more immunologically robust and well fed for the first time. The Expensive Tissue Hypothesis states that the reduction of gut tissue due to greater digestion efficiency through newly diversified microbial populations could have left an opening for the reallocation of nutrients towards the expansion of brain tissue (Aiello & Wheeler, 1995). The correlation of the growth of brain tissue with the reduction of colon tissue in evolving hominids supports this theory as the utilization of both tissues is metabolically expensive (Aiello & Wheeler, 1995). This could all be made possible by the consumption of more nutritionally available foods that underwent natural fermentation. One postbiotic, or rather by product of fermentation is gallic acid. During fermentation, gallic acid is produced after the probiotic hydrolyses of the gallic groups found on phenolic compounds. The higher concentrations of gallic acid result in greater targeting of bitter taste sensing receptors, (i.e. taste 2 receptors, TAS2R; Sterneder et al, 2021). One of the most promising health benefits of gallic acid and fermented products in general is their strong antiradical activity (Jayabalan et al., 2008; Adebo & Medina-Meza, 2020). When assessed for free radical scavenging ability, gallic acid scored highly on the most common laboratory assessments for measuring free radical scavenging (i.e. 2,2-diphenyl-1-picrylhydrazyl, DPPH; Trolox). Researchers indicated that gallic acid is nearly twice as effective at reducing free radicals than other antioxidants, such as caffeic acid, ascorbic acid, and vanillic acid to name a few (Brand-Williams et al.,1995). Fermentation can amplify the health benefits of many products and has the potential to do the same for sorghum.

Fermented Sorghum

As mentioned previously, polyphenol in content in high tannin varieties of sorghum can range from 23-62 mg of polyphenols per gram while other foods traditionally known to be rich in antioxidants such as blueberries, carry only around 5 mg of polyphenols per gram (Burdette et al., 2010). Due to the higher concentration of polyphenols in sorghum compared to other products, fermentation results in greater gallic acid content post-fermentation. The success of specifically sorghum in a fermented state has been only recently explored. The evaluation of traditional dishes utilizing fermented sorghum have been a starting point for the development of novel products. Fermented sorghum is commonly consumed in African countries with dishes such as *Ogi Baba*, a common Nigerian breakfast made from corn or sorghum meal and *Obushera*, a popular fermented sorghum-millet beverage from Uganda. These are both fermented using predominantly lactic acid producing bacteria that have a strong inhibitory effect on enteropathogens (Byakika et al., 2020; Otunba et al,. 2021). Fermented white sorghum specifically has the ability to promote the growth of probiotic bacteria (Garzon et al., 2023). This combination has the as well as the proliferation of certain probiotic properties such as β - Galactosidase that can aid in the management of lactose intolerance (Otunba et al., 2021). Another common condition that sorghum has the potential to address is celiac disease. The combination of a sorghum-based flour with the fermentation practices used in sourdough production have created a superior gluten free bread formula. The use of sorghum and fermentation produced bread with less holes and an overall better crumb when compared to other gluten free options (Schober et al., 2007).

Fermentation also enhances sorghum as it results in an increase in antioxidant effects through DPPH scavenging ability and reduction of reactive oxygen species (Olawole et al., 2019; Banwo et al., 2021). Greater anti-inflammatory effects are seen in fermented sorghum extract when compared to a non-fermented sorghum extract through the inhibition of pro-inflammatory cells that are main drivers of pain and inflammation (Vascular cell adhesion molecule 1; VCAM-1; Cyclooxygenase-2; COX-2; Song et al., 2019). The exposure of raw phytochemicals to fermentation results in novel, small molecule phytochemicals that have more powerful antioxidant, anti-inflammatory and neuroprotective effects (Baciu et al., 2023). These benefits have the potential to aid obese populations with the metabolic pathologies that they are facing.

Obesity

Researchers determined that one of the underlying problems that exacerbate obesity is chronic inflammation. Nearly three decades have passed since obesity has been declared an epidemic by the WHO in 1997. However, it continues to be a rising issue as approximately 340 million adolescents aged 5-19 are estimated to be obese worldwide, while that number grows to 650 million for adults (Haththotuwa et al., 2020). While obesity is commonly associated with the abundance of the upper class, it has been found that obesity has a higher likelihood of occurring in people of a lower socioeconomic standing (McLaren et al., 2007; Anekwe et al., 2020; Benusic & Cheskin, 2021). The most at risk populations consisting of women, African American adults, as well as low-income adults (Anekwe et al., 2020). This is a result of a reduced access to nutritionally fortified foods, exercise equipment, as well as increased stress and anxiety. Food insecurity causes people to rely on low-cost, high-energy foods resulting in over consumption of unhealthy foods. This is reflected as food insecure adults are 61% more likely to be obese than food secure adults (Pan et al., 2012). These populations are also more likely to experience states of heightened stress resulting in metabolic changes that contribute to the generation and maintenance of an obese body type. A heightened state of duress causes glucose to be distributed to give the body the energy to react to a physical stressor. However, in modern society a lot of sources of stress are psychological. This glucose then has nowhere to go and is then stored in the liver and skeletal muscles in the form of glycogen (Tomiyama, 2019). This conclusion has been reinforced by the findings that repeated activation of the stress axis is related to abdominal obesity (Björntorp, 2001). Triglycerides reside within white adipose tissue and create two functional groups. The first is visceral white adipose tissue which causes insulin resistance, inflammation, and diabetes. The second is subcutaneous white adipose tissue which is associated with metabolic improvement and insulins sensitivity due to the fact that it contains partly brown adipose cells that can perform fat burning metabolic processes (Boleti et al., 2023). The inflammation experienced created by these processes could spread to the brain impacting the performance on a cognitive based task in metabolic models.

Neuroinflammation

Obesity has found to be accompanied by chronic, peripheral, low-grade inflammation (Castanon et al., 2015). This pathology can contribute to numerous neurodegenerative disorders. The purpose of inflammation is to activate immune cells in the event of tissue injury or infection. These immune cells are meant to clean away unwanted debris and pathogens while aiding in regeneration (Ahmed, 2011; Onronsky et al., 2022). However, sometime this process does not properly shut off or targets areas that where it is not necessary. When impacting the brain, pro-inflammatory mediators are able to permeate through the blood-brain barrier causing damage to synapses, neuronal death and overall cognitive dysfunction (Lyman et al., 2014). In a juvenile, obese rat model hippocampal function was particularly vulnerable to inflammation in the performance of a Morris water maze. The obese rats saw a delay in spatial reversal learning as a spike in pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha and Interleukin-1-beta in the hippocampus interfered with the memory consolidation process (Boitard et al., 2014). This highlights not only the negative impact a high fat diet can have on the brain but that the degenerative effects are amplified when experience during critical windows of neurological development that is seen in our school aged children. The short-term memory degradation that is seen in models such as this can also be attributed to the spike in prostaglandins that is a result of chronic neuroinflammation (Hein & O'Banion, 2009; Cunningham & Skelly, 2011). The brain experiences disruption and there is an influx of leukocytes traveling in. Based upon the area of the brain impacted by this phenomenon many different pathologies can be seen such as anhedonia, hypersomnia, reduced social behaviors, and cognitive dysfunction (Dantzer & Kelley, 2007; Hein & O'Banion, 2009). This multifaceted phenomenon can be effectively and efficiently studied utilizing swine models that are able to mimic human metabolic syndromes.

The Ossabaw Pig

Sixteenth century Spanish explorers traveled to the Americas with a herd of pigs as a food source. A certain number of these pigs, either escaping on their own or being intentionally released began the well-established colony of Ossabaw swine residing on Ossabaw Island off the coast of the state of Georgia. Due to their limited space on the island the Ossabaws became smaller over time and can be classified as minipigs with the average adult weighing in between 65-154 lbs (Zhang et al., 2021). Eventually, the species came to be known as invasive as they started to diminish the local loggerhead turtle population by eating eggs out of nests. The island population of Ossabaws was reduced to the point of being endangered when a group of scientists lead by Dr. Mouhamad Alloosh and Dr. Michael Sturek of CorVus Biomedical recognized their scientific significance and brought a group of these pigs back to Indiana where they are currently being bred for use in metabolic research. These scientists recognized the significance in the way these pigs store their fat. In order to survive the often-harsh conditions of a small island, the pigs developed the ability to rapidly store fat in times of abundance for use when food is scarce. This phenomenon is comparable to the prediabetic state of a human (Lim et al., 2018). The unique genetics of the Ossabaw minipig make it an ideal model for translational research for a number of metabolic and cardiovascular syndromes (Sturek et al., 2020; Zhang et al., 2021). This unique genetic mutation has come to be known as the “thrifty” phenotype and causes reduced muscle glycogen and a higher level of intramuscular fat deposition compared to other commercial breeds of swine (Cluzel et al., 2022). This allows for a cost-effective induction of obesity and subsequent testing and refinement of weight loss techniques and products such as bariatric surgery (Sham et al., 2014; Simianu et al., 2015). The Ossabaw model is very effective at modeling metabolic syndrome and the impact it can have on cognitive functions.

Cognition

The definition of cognition is multifaceted and subject to a certain degree of interpretation. Therefore, the study of cognition is the struggle to apply a word with an ambiguous framework to neural functions and principles possessing sharp delineations. As if attempting to make a very square peg fit into a metaphysical hole. It is the marriage between Descartes’ “I think, therefore I am” and the use of blood oxygen levels to assess brain function during decision making. In the simplest sense it can be nicknamed the “science of knowing.” It is the way in which the mind perceives, stores, transforms, and utilizes information. It is considered by some to be an antiquated term developed in the times when no species other than humans were considered to be capable of executive function. As the definition of cognition has grown since the time of Darwin so has the framework of what qualifies as a cognitive process. This process: facilitates a higher level of complexity than that of a reflex; requires learning to occur; must depend on reinforced neural pathways and requires access to stored info on models and relationships (Bayne et al., 2019).

These touchstones can be applied to assess behaviors as well as develop new cognitive testing methods for all species. Pigs have demonstrated abilities to reach these benchmarks in numerous ways. Špinka and others (1998) demonstrated that pregnant first parity female pigs (gilts) were able to differentiate between different visual cues assigned to crates that possessed a short (30 minutes) vs a long (240 minutes) confinement time. Pregnant gilts' resting duration increased from 30% to 70%, demonstrating that forced exposure to chronic confinement pen was altered, but over time, they developed a preference for the minimal confinement pen. Based on these results, researchers extrapolated that that the pigs are able to relate external cues with the long-term consequence of either short- or long-term confinement. The decision-making process the pig went through to make their choice of pen meets the previously stated cognitive benchmarks through the use of episodic memory. Episodic memory allows for the pigs to store and recall memories of their previous experiences to continue to make a more informed choice (Dickerson & Eichenbaum, 2010). Conscious recollection supports strong cortical pathways throughout the prefrontal cortex and hippocampus (Moscovitch et al., 2016; Sugar & Moser, 2019).

As pigs emerge as a model for cognitive research, it is essential to have their true abilities confidently recognized. The possibilities for pigs on pharmaceutical studies would be significant as pigs are, phylogenetically, much closer to humans than the commonly used rodent model (Gieling et al., 2011; Walters and Prather 2013; Tang and Mayersohn, 2018; Lunney et al., 2021; Cupitra et al., 2023). More specifically, the minipig has already been used in biomedical research reaching back over the past two decades (Vodecka et al., 2005; Nunoya et al., 2007; McAnulty et al., 2011). However, within the many studies that make huge strides in the fields of comparative medicine and pharmacology, you don't see any extensive behavioral data collection (Yu et al., 2020; Denner et al., 2021; Kenchegowda et al., 2021; Kuzma et al., 2021; Wang et al., 2021; Li et al., 2022). The pig could be a preferred model for pharmaceutical testing as they are neurologically similar to humans (Sauleau et al., 2009; Conrad et al., 2012; Weickenmeier et al., 2018; Simchick et al., 2019).

The ability to uptake an operant task through conditioning allows for the assumption that the pig can prioritize memories that they have assigned a certain amount of importance based on a perceived outcome i.e. the procedure to complete a feeding task (Marino and Colvin 2015). The hole board test has been particularly well adapted to assess cognition in swine. Originally designed for rodents, the hole board test consists of 4 x 4 matrix of compartments that food can be hidden within. The animal must utilize reference and working memory to learn the pattern in which the holes are baited. In Grimberg-Henrici and others (2016), researchers assessed the impact of environmental enrichment on the performance of a hole board task. This study utilized 10 pairs of Duroc x Terra x Finnish Landrace pigs where one half of the pair was assigned to a barren pen while the other was assigned to a larger enriched pen consisting of straw, a rooting area, and four different toys. The enriched-housed pigs had fewer visits to unbaited holes over time indicating superior reference memory. The enriched-housed pigs also had a better working memory as they made fewer return trips to previously baited holes. Improved performance of the barren-housed pigs over the course of habituation is noted as well. The author suggests that this is due to the test itself becoming enrichment that mitigated the negative effects of barren housing. This study highlights the cognitive competency of pigs as well as the impact environmental welfare can have on performance and the potential for this test to assess that discrepancy. The refinement of these tests will allow for more analysis of cognitive and behavioral welfare of animals with the option of being exposed to a set of experimental factors. To be able to effectively monitor and understand the cognitive wellbeing of production animals would mean the opportunity to improve animal husbandry in both biomedical and production swine. While cognition covers how the subject is able to perform a task, there are additional mechanisms contributing to the why they are performing it and the reinforcing nature of the reward system.

Cognition and the Reward System

Motivation based behaviors and task competition can be broken down into neurochemical components. One of the smallest but most integral being the monoamine neurotransmitter dopamine (DA). Both a neurotransmitter and a hormone, dopamine falls into the catecholamine category of neurotransmitters along with the likes of epinephrine and norepinephrine. The significant role motivation plays along the "reward pathway" includes the reinforcement of the cycle motivation. At least eight dopaminergic pathways have been identified within the brain, however, the pathway most involved in motivation behaviors is the mesolimbic pathway (Lewis et al., 2021). The mesolimbic pathway transports dopamine from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and amygdala. The VTA can be found in the midbrain and is known for a role in motivational behaviors. Rewarding stimuli sich as food, sex and drugs result in a rise in dopamine within the NAc (Ikemoto et al., 1999; Di Chiara et al., 2007; Arias-Carrion et al., 2010). This occurs due to the innervation of dopamine projecting neurons from the VTA that make up the mesolimbic pathway (Salamone et al., 2012; Blaess et al., 2020).

The nucleus accumbens is located within the ventral medial portion of the striatum and is the hub for motivation and cyclical reward behaviors (Salamone et al., 2005; Salgado, 2015). While the full scope of activation of the NAc is still poorly understood, researchers postulate that the rapid increase of dopamine the NAc receives from the VTA is a main driver in the repetition of a task due to the euphoric effects (Floresco, 2015; Klawonn & Malenka, 2018). Dopamine continues to the amygdala which serves as an integral part of the limbic system. The amygdalae are two symmetrical paired structures, found on either side of the medial temporal lobe and are known for being the epicenters for emotional processing. The fact that the amygdala is a stop on the mesolimbic pathway shows support for its association with emotional based reward behaviors (Blaess et al., 2020). The other major dopaminergic pathway within the brain is the mesocortical pathway. As in the mesolimbic pathway the mesocortical dopaminergic response originates in the ventral tegmental area (VTA). From there action potentials project to the prefrontal cortex (PFC; Reynold & Flores, 2021). The prefrontal cortex is a complex area of the brain known for primary involvement in executive function. Having been the most recently evolved area of the human brain, the prefrontal cortex is responsible for all higher-level cognitive functioning (Arnsten, 2009; Kolk & Rakic, 2022). The network of neurons within this region of the brain can retain information even without the correlating environmental stimulation (Fuster, 2015). This ability is what makes the concept of working memory possible.

The initial location of stimulation influences the dopamine pathway. The more well-known process of phasic dopamine release occurs quickly upon the prediction of an impending award or even the presence of an unexpected one. This results in a large amount of dopamine flooding into the synapse between dopamine neurons and those found in the nucleus accumbens. In contrast to this event, dopamine is slowly and consistently being released in low volumes around the synapse creating a level of extracellular dopamine that remains too low to stimulate the main receptors on the cells of the nucleus accumbens (Ikemoto et al., 2015; Berke, 2018). However, dopamine can bind to auto receptors on the spectific VTA neuron in order to limit the phasic release of dopamine. Tonic dopamine release is believed to be more useful in the development of reinforced learning (Beeler et al., 2010).

Motivation could be largely dependent on extracellular levels of dopamine (Volkow et al., 2017). Therefore, the behavioral objective measurements of motivation are likely to reflect concentration. Specifically, dopamine levels have impact how much repetitive reward-based behaviors are performed in relation to a task (Aberman and Salamone, 1999; Beeler et al., 2010; Foo et al., 2021). This potential discrepancy in dopamine levels could result in the differentiation of being either a freeloader or a contra-freeloader. Dopamine signaling is heavily involved in hippocampal plasticity and the process of long-term potentiation in relation to spatial memory (Speranza et al., 2021). These neurochemical differences could impact task performance on an individual and group level through the process of contrafreeloading.

Contrafreeloading

The procedural concept of task completion is one that spans many species that are capable of a complex level of thought. Contrafreeloading has also been studied in a wide variety of animals (De Jonge et al., 2008; McGowan et al., 2010; da Silva Vasconcellos et al., 2012; Schepisi et al., 2016; Rosenberger et al., 2020; Smith et al., 2021). The term contrafreeloading refers to the phenomenon that animals often prefer a reward that they must work for over one that is freely available (Osborne, 1977). The satisfaction that results from the successful execution of a task has been explored in swine (Young and Lawrence, 2003; De Jonge et al., 2008). De Jonge (2008) studied this concept by exposing 21 piglets to a "*Conditioned Place Preference Task*". Researchers put the pigs in a pen with two different ways to acquire a reward (chocolate raisins). They found that the piglets had a significantly higher preference percentage for consuming raisins that they had to look for in the hay over raisins that they could get much quicker out of the trough. The same study also assessed the exposure of 13 piglets to a food reward immediately after a conditioned stimulus (three white light flashes from a 60W bulb located above the feed trough). In one environment the light flashes right after the piglet was placed in the testing arena to announce the delivery of 3-5 chocolate raisins. In the experimental compartment, the food reward was still delivered after the flashing lights, but at an increasing delay of between 8 and 14 seconds. The implementation of the delay allows for the development of anticipatory behaviors. Pigs possess the cognitive ability to anticipate events once they are conditioned (Dudink et al., 2006; Imfeld-Mueller et al., 2011; Mahnhardt et al., 2012). Anticipatory behaviors specifically are positively correlated with reward sensitivity (Jensen, 2017). It can be postulated that the ability to perform these behaviors along with those found in an appetitive refractory cycle is indicative of a more positive affective state. The assessment of affective states while not extensively explored, is essential to assessing welfare in any kind of animal. De Jonge (2008) shows that the anticipation and prediction of gaining the reward after the delay of labor was a more powerful reinforcer than the simple acquisition of food alone, as the pen that experienced the delay had a higher preference percentage as well. Contrafreeloading is often identified in the feeding behaviors of animals. This author brings up the point that pigs are more inclined to perform contrafreeloading when the task is more akin to their natural behaviors such as rooting. Other publications such as Young and Lawrence (2003), did not show as clear of a distinction in the performance of contrafreeloading in an operant conditioning task such as a lever push task adhering to either a fixed reinforcement schedule or a variable reinforcement schedule. These researchers utilized six Large White x Landrace female pigs. Each pig was housed individually, and their pens contained two identical food bowls from a modified calf feeder. Mounted above each bowl was an *"operant manipulandum"* (lever). To operate the manipulandum the pig had to push against the metal panel with enough force to trigger the reward. 30 grams of food was dispensed upon activation based on the operant schedule determined by the experimenter. One bowl and panel were active while the other was not. Pigs did show increased motivation within this methodology considering that food was withheld overnight. Young and Lawrence (2003) found that there was no significant level of contrafreeloading within their task. They concluded that the degree of contrafreeloading expressed is dependent upon experimental conditions. They determined the level of contrafreeloading performed by the pigs to be negligible and found no significant difference in performance when using a fixed vs variable reward schedule. The authors suggest that the higher the degree of domestication the less likely the animal is to perform contrafreeloading behaviors. This can suggest that since the pigs did not perform a lot of contrafreeloading behaviors within this context that it is not an important to them overall. However, results like this are nuanced as environment must be carefully considered as to how much of an opportunity the pigs were given to perform such behaviors. De Jonge (2008) provided a more natural context for the pigs to express contrafreeloading behaviors and had a lot more data to support the opposing argument.

Assessing contrafreeloading within the context of a cognitive feeding task allows for the evaluation of its impact on social dynamics. One pig will likely be more inclined to complete the task than the other. The former being a contrafreeloader and the latter being branded a freeloader. This is important to consider from a production standpoint as it can have an impact on caloric allocation and overall performance. There is also the potential of a correlation between being either a contrafreeloader vs a freeloader and the propensity to be dominant or submissive in a binary social hierarchy. The relationship of a freeloader and a contrafreeloader can be a peaceful one within the context of our task as the latter one often pushes both paddles to receive their reward and lets the freeloader have one bowl of feed to themselves. This highlights that the contrafreeloader likely enjoys the process of completing the task as well as favors it over the possibility of sparring with their pen mate.

The dominant pig is the one that will likely have control over a situation and the resource of value that is involved within that situation. If a contrafreeloader is in control of a task that involves a resource as valuable as food, then one can deduce that the contrafreeloader is the dominant pig in the dyad. However, the reaction of the subject that does not perform the task needs to be assessed before assign a role. For example, the non-performing pig could be a subordinate that is simply forced into accepting whatever scraps are left by the dominant pen mate. However, noting the high level of cognition that pigs can achieve it is safe to assume that they can function within complex social situations. The subordinate orintermediate pig could see the formidable opponent that their pen mate poses and strategize that a better use of their energy would be to comply with the dominant pig and eat whatever is left over, thus becoming a freeloader. If they are truly subordinate or just a savvy intermediate would be determined on a case-by-case analysis of social interactions.

There is also the potential for cognitive differentiation between more dominant and more submissive pigs and how social status can impact their decision making. Within the context of a cognitive task, a contrafreeloading strategy has the greater potential for utilizing the neurochemical pathways that innervate the prefrontal cortex (Funahashi & Andreau, 2013; Fuster, 2015; Friedman & Robbins, 2022). A dominant pig that would rather utilize a cognitive freeloading strategy by utilizing physical size and force to achieve their goal, could be making more impulsive decisions that require less cognitive function and more physical work. This decision is impacted by the social relationship and behaviors displayed with conspecifics.

Social Behaviors

Pigs are social animals (Jensen, 2002). If given the opportunity they tend to form groups in a size of five to eight. Pigs, like multiple other species, are very adept at discriminating between familiar and novel herd mates (Gieling et al., 2010; Marino and Colvin, 2015). They also carry a strong preference for pigs that are already familiar to them (Camerlink et al., 2014). This can make it difficult to integrate new pigs into an already group housed herd. Pigs operate using a social hierarchy formed through short-lived conflicts as the pigs figure out who is dominant and who is submissive. After a solid hierarchy is formed, many agonistic interactions can be solved by simple avoidance behaviors if the other pig was convinced to take on the role of subordinate. Pigs then become habituated with each other as they gain greater comfort within the roles within the group (Puppe et al., 2008). Due to the current push for group housing, it is likely that pigs will come into contact with novel conspecifics and social hierarchies will have to be reestablished. Injuries due to fighting are common and can result lesions, infection, lameness, and an increase in stress activation along the HPA axis (Hypothalamic Pituitary Adrenal axis; Boyle et al., 2002; Ison et al., 2016; Morgan et al., 2018). All of which negatively impact production efficiency (Heinonen et al., 2013). These behaviors specifically are likely to continue in group-housed systems especially in the presence of food. Pigs will always form groups given the chance, but sow groups in particular experience fewer agonistic interactions when compared to weaning and growing aged pigs (Puppe et al., 2008; Parent et al., 2012). Younger pigs are likely to put themselves in these agonistic interactions as a way to gain experience socially within the herd and to potentially increase their status. The regulation of these social behaviors is integral as the sensitive period of the brain in pigs occur from 2 to 24 weeks of age (Conrad et al., 2012). The presence of social experiences during the critical windows allows for faster and more robust brain development (Fil et al., 2021). Social interactions also allows for great emotional regulation and thus, improved health and welfare of the group (Puppe et al., 2008). Gestating sows were also shown to have a higher degree of temporal stability within their hierarchy over the course of their pregnancy when compared to growing females (Parent et al., 2012). Females overall were able to adapt to much more stable social hierarchies than males (Tong et al., 2020).

Social behaviors are highly regulated by both the prefrontal and insular cortices (De Bruin ,1991; Lamm & Singer, 2010). The prefrontal cortex is essential to the process of goal-orientated social behavior. This is achievable through adaptive social norms that the prefrontal cortex is able to group together based on past experiences (Barbey & Grafman, 2011). The utilization of these frameworks for future use plays a key role in social inference and allows for the potential for cooperative behaviors or conflict to form. The insular cortex is a lesser-known lobe of the cerebral cortex located within the Sylvian fissure. Some of the key functions of the insular cortex include gustatory processing, reward behavior, pain mechanisms, and auditory functioning (Kortz & Lillhei, 2023). The insular cortex also plays a role in social emotions as MRI studies have shown significant stimulation of this area during feelings of compassion, admiration, and love (Lamm & Singer, 2010). As the insular cortex produces these feelings the prefrontal cortex helps these details to develop the frameworks of adaptive social behavior. Within the context of social animals this allows for detection of these feelings and patterns in conspecifics and decide if they would like to interact or cooperate (Rogers-Carter et al., 2018). Social behaviors go hand and hand with feeding behaviors as individual versus pair housed pigs will approach mealtime differently.

Feeding Behaviors

The classification of pigs as freeloaders and contrafreeloaders is accompanied by a third intermediate state that can be defined as cooperation. The pigs within these binary hierarchies can take on the roles of dominant, submissive, or intermediate. This allows researchers to draw correlations between the pigs labeled as such and their likelihood of becoming a freeloader, a contra-freeloader, or a cooperative within a testing environment. Each of these dynamics can produce a stable or unstable relationship. This information is very valuable for a fully fleshed out assessment of cognitive performance while under the influence of a social dynamic. Cooperation of cohorts can provide various evolutionary advantages such as reduced risk of predation, successful foraging/hunting, assistance rearing offspring and thermoregulation (Marchant-Forde, 2010). The development of these relationships is highly variable on environment.

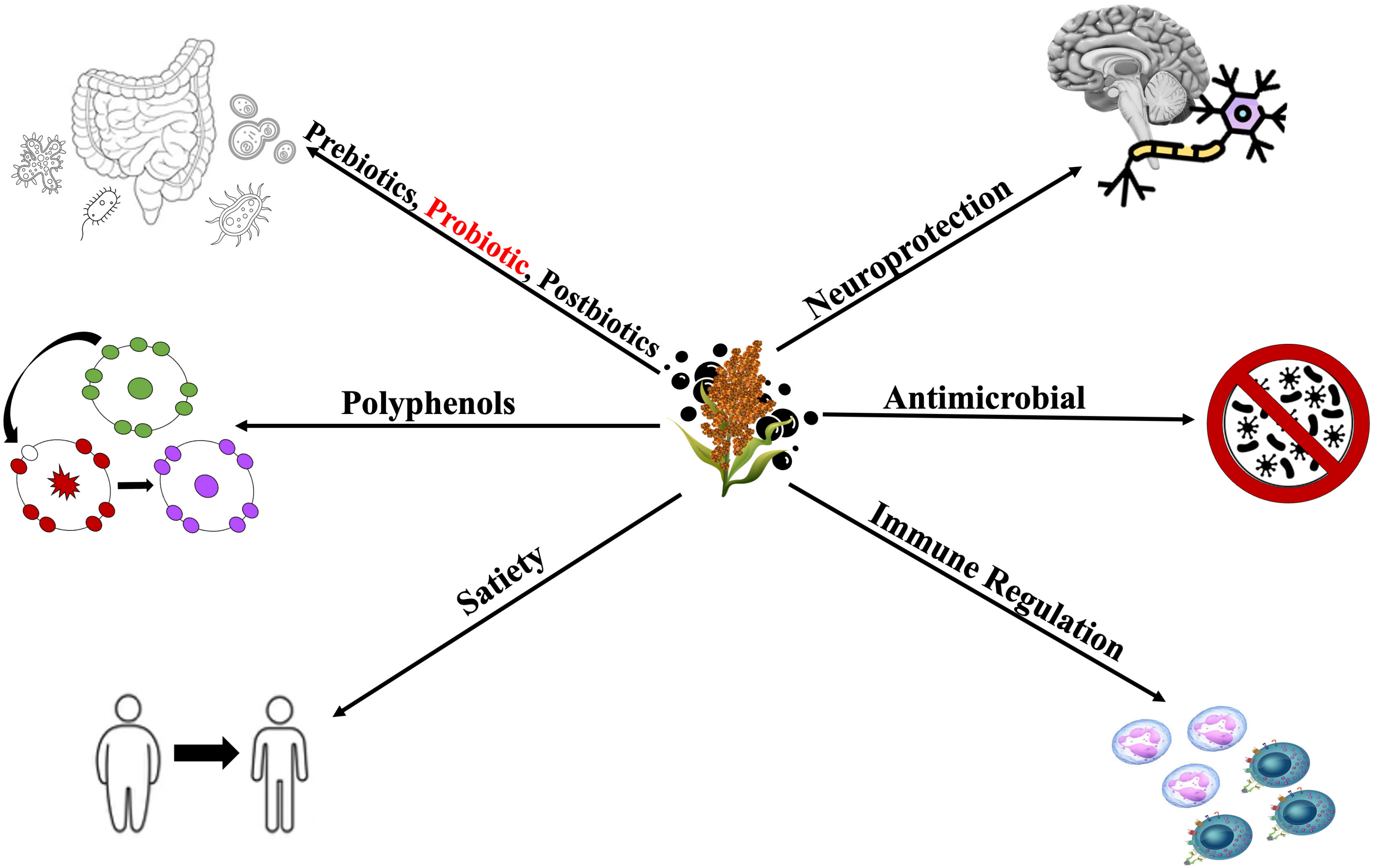
Housing and care of commercial swine has been brought to the forefront in recent years as welfare concerns over food animals rise. Individual stalling for boars as well as gestation crates for sows are commonly utilized in commercial pork facilities as a way for producers to precisely control the reproduction and nutrition of their herd while cutting down on undesirable behaviors such as fighting (McGlone & Salak-Johnson, 2008). This isolated method of housing possesses many welfare concerns ranging from physical, such as confinement injuries such as pressure sores and ulcers (Boyle et al., 2002) to behavioral, like the development of stereotypical behaviors due to lack of mental and social stimulation (McGlone, 2013; Zhou et al., 2014). However, group housed systems have their own set of challenges such as increased injuries from fighting as well as unequal nutrition as the result of the formation of social hierarchies. New technology and procedures are needing to allow for a sustainable departure from individual housed systems. The use of precision feeding technology can address the nutritional discrepancies caused by group feeding. In order for this to occur the feeding behaviors of swine must be reviewed. The introduction of precision feeding technology, more commonly seen in cattle, could be a solution to some of these issues. Studies conducted on feeding patterns of swine utilizing precision feeding equipment show that the overall habits of pigs do not significantly alter their feeding behaviors in terms of number of meals, duration of meals, time between meals, feed consumed and feed consumption rate (Andretta et al., 2016). Various studies have assessed the impact that housing status can have on how pigs feed. Dominant status correlates with a higher average daily gain and a greater number of expressed feeding behaviors. This results in a correlation between feeding frequency and the frequency of aggressive acts (Vargas et al., 1987). Thus, the presence of competition having a stimulatory effect on certain aspects of feeding behaviors. This is also shown as Bornett and others (2000) evaluated the differences in feeding patterns in boars that were housed individually before being moved to a group-housed system. The pigs began in an individually housed setting where they expressed a high rate of feeder visits with a lower rate of consumption. This is likely attributed to a lower level of mental stimulation and lower motivation produced by isolated, ad libitum feeding. Once group-housed, the boars had a decreased frequency of feeder visits but an increased duration of feeding and overall rate of consumption. They also found that the frequency of aggressive behavior decreased over time suggesting a development of social cohesion.

While this phenomenon can result in a decrease in feed intake for certain members of the group, similar deficits can be found in single-housed pigs. Decreased intake in these settings has been shown to be remedied by having access to viewing neighboring pigs consume feed (Hsia and Wood-Gush, 1983). This is known as the theory of "social facilitation" and can induce more simultaneous feeding behaviors that is often desired in group housed settings.

Conclusion

Obesity and related comorbidities are a huge problem facing society today. The development of a novel fermented sorghum kombucha could present a fortified and cost-effective delivery system for the nutrients that so many people are missing from their diets. The use of the Ossabaw pig model for the maze and feeder tests specifically offer efficient and ethical means to measure performance with the opportunity to apply numerous experimental conditions. Further repetition and validation of these procedures is currently underway on a larger scale and the potential for their use is far reaching. The use of these techniques can aid in the testing and development of many novel products as it is doing for the fermented sorghum tea that has the potential to address many health and satiety concerns in production animals and humans alike.

Figure 1.1. Fermented sorghum products are rich in probiotics, as well as prebiotics which act as food for the probiotics, and postbiotics, which are the by-products of the metabolic fermentation process. Sorghum is also very rich in polyphenols which have strong antioxidant effects as they are effective free radical scavengers. The increased satiety is due to the alteration that proteins experience during the fermentation process resulting in the starches digesting at a slower rate thus, regulating postprandial glucose and curbing cravings for a longer time. Secondary benefits stemming from the probiotic potential of fermented sorghum are immune regulation and anti-inflammatory activity, antimicrobial effects, and suppression of enteropathogenic bacteria. Lastly, and the least explored is the potential neuroprotective benefits of polyphenols and their amplification through the fermentation process.



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Chapter 2 - Adult Ossabaw Pigs Prefer Fermented Sorghum Tea Over Isocaloric Sweetened Water

Abstract

Ossabaw pigs (n=11; 5-gilts, 6-barrows; age 15.6 ±0.62 SD months) were exposed to a 3-choice preference maze to evaluate preference for fermented sorghum teas (FST). After conditioning, pigs were exposed in 4-sessions to choices of white-FST, sumac-FST, and roasted-sumac-FST. Then, pigs were exposed in 3-sessions to choices of deionized-H2O (-control; avoidance), isocaloric control (+control; deionized-H2O and sucrose), and blended FST (3Tea; equal portions white, sumac, and roasted-sumac). When tea-type was evaluated, there were no clear preference-behaviors for tea-type were observed (*P* >0.10). When the 3Tea and controls were evaluated, pigs consumed minimal -control (*P* <0.01; 18.0 ±2.21% SEM, respectively), and they consumed great but similar volumes of +control and 3Tea (96.6 and 99.0 ±2.21% SEM, respectively). Likewise, head-in-bowl duration was the least for -control, but 3Tea was the greatest (*P* <0.01; 5.6 and 31.9 ±1.87% SEM, respectively). Head-in-bowl duration for +control was less than 3Tea (*P* <0.01; 27.6 vs. 31.9 ±1.87% SEM). Exploration duration was greatest in the area with the –control (*P* < 0.01; 7.1 ±1.45% SEM, respectively), but 3Tea and +control exploration were not different from each other (1.4 vs. 3.0 ±1.45% SEM, respectively). Regardless of tea-type, adult pigs show preference for FST, even over +control. Adult pigs likely prefer the complexity of flavors, rather than the sweetness alone.

Keywords: Sorghum; Fermented; Tea; Porcine; Taste; Sensory; Ossabaw; Swine; Pig; Flavor

Introduction

Sorghum is a potentially more sustainable crop than other grains due to the plant's resilience to dry, hot climates (Chipanshi et al., 2003; Chadalavada et al., 2021; Warsame et al., 2022). Therefore, new, palatable products need to be developed to im-prove both food availability and nutrition-security. Compared to other cereal grains, sorghum may provide more nutrition-security because of greater concentrations of phenolic compounds (Xiong et al., 2019; Xu et al., 2021). Phenolic compounds possess significant antioxidant abilities that modulate chronic inflammation (Punia et al., 2021), prevent cancerous cells proliferation (Arora et al., 2019), and may even improve satiety during weight loss programs involving tea-based products (Li et al., 2020).

Nonetheless, sorghum strains with high concentrations of phenolic compounds (e.g., red or black sorghum) may contribute to increased bitterness in products because of the associated in-creased tannins (Xu et al., 2021, Soares et al., 2013, Soares et al., 2020). Furthermore, when sorghum is heated, it decreases protein bioavailability in cooked sorghum, which may be caused by phenolic compounds binding to proteins (Xu et al., 2021; Duressa et al., 2018). These drawbacks may be remedied through the process of fermentation. (Kazanas & Fields, 1981; Schons et al., 2012; Rodríguez-España et al., 2022). For example, sorghum can be cultured to produce potentially beneficial by-products and increased protein bioavailability and the fermentation of the liquid may be palatable to adults (Elkhier & Alraheem, 2011; Sorour et al., 2017; Bwamu et al., 2022).

For this current project, fermented sorghum-based tea was created because of the potential to have high concentrations of antioxidants and probiotics, thus providing a nutrition-secure beverage. Although sorghum-teas and fermented-teas originated in northeast China around 220 B.C. (Kapp & Sumner, 2019; Sun et al., 2020), fermented teas are new to the other countries. The U.S. Kombucha is a recent example of a commercialized fermented tea, which is produced by first seeping black, green, or oolong tea leaves, then, adding sugar and a symbiotic culture of bacteria and yeast (SCOBY; Kapp & Sumner, 2019; Xu et al., 2023). These same methods could be applied to sorghum, but with Kombucha-formula's, the fermentation results in sour flavors and astringent mouthfeel (Bwamu et al., 2022; Xu et al., 2023). A major influence of kombucha flavor is the organic acid content produced by the microorganisms within the SCOBY. Specifically, malic, gallic, and lactic acids contribute to added sour flavors and dry mouth feel, which also are associated with red wines even though these compounds contribute to the antioxidant properties of fermented beverages (Xu et al., 2023; Badhani et al., 2015; Sterneder et al., 2021).

Although the final product will be primarily marketed for humans, the current authors anticipate that the byproducts of producing fermented sorghum may also improve health among of commercially produced pigs, particularly among adult pigs that are fed restricted calories to avoid obesity. However, previous research shows that pigs may reject sour or bitter flavors. Nevertheless, most preference research has been conducted on either young commercial pigs at weaning, or ad libitum fed sows that have inappetence after parturition (Kennedy & Baldwin, 1972; Nelson & Sanregret, 1997; Kittawornrate & Zimmerman, 2011; Guzmán-Pino et al., 2019). There is a lack of research publications on flavor preference in adult pigs that must be limit fed.

Therefore, the objectives of this study were: 1) Assess adult pig preference among three types of formulated sorghum teas, and 2) compare the preference or avoidance behaviors among choices of blended fermented sorghum tea, isocaloric sugar water, and negative control water.

The hypothesis was that adult pigs would prefer the sorghum tea with the least amount of gallic acid and the isocaloric control (sugar water) over a blend of fermented sorghum teas.

Materials and Methods

Animals and Housing

This study followed the guidelines set forth by the Guide for the Care and Use of Agricultural Animals in Agriculture and Teaching (CorVus IACUC #4745). Sample size calculation was completed using variables from a preference study in another line of Iberian minipigs (Gautier et al., 2019). From this research, volume intake, duration of head-over-bowl, exploration, and consumption were the main variables considered. Then, sample size calculations were made using veterinary epidemiology methods previously described (Martin et al., 1987). The calculator was set to an alpha of 0.05 and the power (1-beta) to 95. Each measure's standard error was entered and the desired magnitude of difference between sexes for each measure was entered. The average sample estimation was 5 pigs per sex. Behavioral tests and pig-types from this literature were not an exact match for this experiment, therefore an additional pig per sex was requested, resulting in 12 subjects. After IACUC approval pigs were carefully selected for our project. Future studies aim to better understand the health benefits and behavioral changes associated with consumption of sorghum tea, especially among adult subjects. The Corvus research herd had up to 200 pigs, mainly breeding dams, sires, and offspring. Criteria for this project were healthy, lean adult pigs that had never reproduced and were over 8 months old. Therefore, we were limited to virgin adult female pigs and barrows that already had familiarity with each other for ease of acclimation into new pairs and the experiment facility. The animal portion of the project was conducted between May and June 2022. Twelve adult Ossabaw gilts and barrows (age 15.6 ± 0.62 SD months; 53.5 ± 0.62 kg body weight) were pair-housed by sex at indoor, climate-controlled facilities (21°C; CorVus Biomedical, LLC, Crawfordsville, Indiana). Artificial lighting was a 12-h cycle, with lights on from 0800 to 2000 h. One month prior to the start of the experiment, pig pairs were randomized into one of six pens that were stratified by sex (gilts and barrows). Pens provided 14.92 m2 of free space with plastic slotted flooring (Maxima Sow Slat Floor, Greenfield, WI USA). The gating for the pens were 94.0 cm tall and custom made with vertical standard stainless bars (Thorp Equipment, Thorp, WI USA). Two feeders (S3 Series Dry Sow Feeder, Crystal Spring Hog Equipment Agathe, Manitoba CA) were provided on each side of the pen with a drinker (ad libitum well water; QC Swine Water Cup System, Delphi, IN USA) next to one of feeders. Environmental enrichment consisted of two combined stainless-steel chains, with one link fastened near the drinker. Pigs were fed according to National Research Council (NRC) standards. Pigs were fed a total of 750 g feed per day to maintain, a healthy, lean body type (15% Crude Protein, 3% Crude Fat; 5% Fiber; DuMOR Hog Grower, Tractor Supply, Brentwood, TN USA). Pigs were fed at 0900 h and each daily testing sessions took place one hour later. Pig pairs were randomly assigned to pen using the RAND function of Excel (Microsoft Excel, Version 10; Redmond, WA). Then, individual pigs within each pen were randomized for exposure order to the testing arena.

Formulation of Solutions

Three varieties of sorghum (Nu Life, Scott City, KS) were used for this study: raw-white, raw-sumac, and roasted sumac strains. To create the sorghum tea (Figure 2.1), commercially available (for human consumption) sorghum was steeped in sterile water at room temperature for 16 hours. Then, the supernatant was recovered by filtering and brought to a boil before sugar was dissolved (Figure 2.1). Once boiled, 2 cups of supernatant (473 mL) were added to a jar with 1 cup of sugar (200 grams) and 8 cups (1892 mL) of deionized water. Afterwards, the solution was cooled to room temperature, and inoculated with a commercially available culture (Symbiotic Culture of Bacteria and Yeast, SCOBY; The Kombucha Shop, Madison, WI USA). Then, the solution fermented for 7 days at room temperature. Fermentation was then stalled by refrigeration. For this current experiment, the solution was refrigerated for 72 h before use. The pH of the fermented solutions were monitored and averaged 3.12 ±0.12 SD. An isocaloric (0.288 calories per mL), positive control (+control) was created with sugar (sucrose) and boiled distilled water. The negative control (-control) was distilled water only. Each batch of solution was sampled and frozen at -20 ºC for gallic acid analyses. Gallic analyses were determined using the Folin Ciocalteu Method as described previously (Singleton & Rossi, 1965; Jayaprakasha et al., 2001; Singh et al., 2002; Dhillon et al., 2021).

Preference Testing

A commercially available, 3 compartment (i.e. pod) preference maze was used for the preference test (3-PodShape, by Hulbert and ShapeMaster, Odgen, IL USA; Figure 2; VideoS1). The three compartments (pods) were made of plastic with non-slip floor mats. On the mid-dle corner of each pod, a small bowl (0.59 L) was fastened over a 1.9 L bowl (stainless steel; Snap'y Fit, Midwest Homes for Pets, Muncie, IN USA). The smaller bowl was used to offer 100 mL of each solution; the large bowl was used to collect spillage. The maze was set up at the end of the facility, and two gates (described above) were used as the entry way (Figure 2.2). A standard pig board was used in the main aisle to shut the subject in the maze (Figure 2.2). A camera (GeoVision Model GV-EBL4702-2F; Geovision, Irvine, CA USA) was placed at a 90° angle over each pod and the entrance area for a total of 4 cameras over the testing arena. A customized NVR recorded each session continuously at 30 frames per second at a video resolution of 1080 p.

In real time, one re-searcher recorded the time of entry and order of entry in each pod. After the session, residual solutions were collected from every bowl and volume refused was measured and recorded using a 25- or 100-mL graduated cylinder, then volume consumed was documented as a percentage.

Prior to experiments, pigs were acclimated and conditioned in pairs twice and then individually to properly navigate the maze and 100 mL of +control was available in each pod of the maze (Figure 2.2). Pigs then were conditioned to predict that there is at least one bowl with desired +control (100 mL). This method causes pigs to explore all 3 options at least once (Nofre et al., 2002). The location of +control was randomized during this conditioning phase (Figure 2.2). For the first experiment, each pod randomly contained one of three types of teas. Experiment 1 consisted of 4 identical sessions with the placement of tea-type randomized. Four sessions were required because the initial trial was the first time that pigs were ever exposed to fermented sorghum teas and therefore, novelty responses could be considered. Each session in experiment 1 consisted of a pod containing one of three varieties of sorghum tea on a rotating basis. The personnel caring for the animal were blinded to the treatments, while the personnel conducting the animal portion of the experiment were provided solutions that were labelled with arbitrary numbers and codes.

For experiment 2, three equal parts of the teas from experiment 1 were combined (3Tea; equal portions of white, roasted, sumac) and were tested against the positive and negative control. Due to the pigs already being familiar with the fermented tea and controls, only 3 identical sessions were conducted. For each pig and each session, the placement of solutions in the pods were randomized. For both experiments, containers were marked with generic numbers and stickers to improve blindness of the observers, although the tea was obviously a darker color than the control solutions. Control solutions were not dyed with food coloring because food coloring has flavor and pigs use their olfactory senses for preference over vision and food coloring may have additional flavors that pigs can detect.

Video Behavioral Quantification

All videos were collected onto a single drive within the computer system. The videos from the 4 cameras were merged into one file and the Geovision codec was converted into a standard H264 into AVI format. The videos were then manually coded for behaviors using the specialized time stamping (coding) software for interpretive, quantitative analyses (Observer 11.3; Noldus Observer Leesburg, VA, USA). An ethogram with mutually exclusive behaviors was created using previously defined ethograms (Hulbert et al., 2019). Subsequently, a coding system was made within the software. The behaviors included spatial x structural behaviors. Spatial behaviors were defined when the first two legs crossed the threshold (dotted line in Figure 2.2) and included: entrance, left-pod, middle-pod, and right pod. Structural behaviors included: head-over-bowl (bowl), exploratory behaviors (i.e. non-nutritive oral behaviors, NNOB; described previously; Hulbert et al., 2019), and all other, non-oral and non-exploratory behaviors. Three macros-based keyboards (X-Keys; Engineering, Williamston, MI USA) were programmed to correspond with the behavioral codes in the ethogram. Three trained ethologists coded the same eleven videos within the software. A Cronbach's reliability test was performed for the 3 observers and 11 samples, and mean alphas were 0.94 ± 0.115 SD and 0.93 ± 0.061 SD for duration and frequency, respectively. After the agreement test was performed, each trained ethologist was randomly assigned up to 4 pigs to code every session. Ethologists were blinded to the arm treatments for the second conditioning phases, and the two treatment phases and only coded for generic spatial behaviors (e.g. left, middle, right).

Statistical Analysis

Statistical analyses were conducted using SAS version 9.4 (SAS, Cary, North Carolina/USA, SAS Institute Inc.). Data were first transposed in SAS from left, middle, right to correspond to the different choices (supplementary SAS code; S2TransposeCode). A linear mixed model for repeated measures was applied (Restricted-maximum likelihood; ANOVA). Included were the random variable of pigs and the fixed effects of choice (tea-type or control added to pod), expo-sure session (Exp), and the interaction of choice and exposure and random effect pig were fitted. The covariance structure for both experiments was compound symmetry. The outcome variables included consumption, duration percentage, latency percentage, and rate/number per min. The data was analyzed for normality by Shapiro-Wilk. Consumption data were not normally dis-tributed with and without transformation, therefore they were evaluated using the non-parametric Kruskal-Wallis test. All other behavior data were square root transformed. Least-Squares means for consumed volume, duration, latency, and rate LS means were assessed. Pairwise comparisons were carried out using the two-sided test; The Tukey-Kramer method was used to adjust for multiple comparisons. A treatment difference of $P \leq 0.05$ was considered significant.

Results

General Results

The fermented tea types had the following gallic acid concentrations (µg/mL): white, 34.57 ±0.47 SD; sumac, 140.41 ±0.41 SD, and roasted sumac, 133.35 ±0.81 SD. The blended tea (3Tea) had a gallic acid mean of 120.70 ±11.57 SD µg/mL. During the conditioning phase, one gilt passed away from unknown reasons (necropsy completed by veterinarian and cardiologist) and did not complete experiment 1 and 2.

Experiment 1

No choice by exposure interactions were observed among any variables ($P > 0.10$; Table 2.1). There was a decrease in consumption volume in the first exposure when compared to later exposures ($P = 0.005$; Table 2.1). Regardless of tea type, pigs spent the most amount of time performing exploratory behaviors in exposures 1 and 2 ($P = 0.029$; Table 2.1). Gallic acid intake was the least for the white tea type, and sumac tea type was the greatest, followed by roasted sumac tea type ($P <0.001$; Table 2.1). The rate at which the pigs performed non-oral behaviors was the lowest during the first exposure ($P = 0.003$; Table 2.1). Pigs performed non-oral behaviors the most ($P = 0.050$; Table 2.1; Figure 2.3) when they were exposed to sumac tea-type, and the least when they were exposed to the white tea type. The pigs checked the bowl at the lowest rate per minute in the first and second exposure. There tended ($P = 0.060$; Table 2.1) to be a greater latency to express exploratory behaviors during the first exposure.

Experiment 2

Consumption volume was the least for the negative control compared to the positive control and 3Tea ($P < 0.001$; Table 2.2). There was a choice by exposure interaction effect; pigs spent the most time in pods containing the 3Tea blend after the first exposure in experiment 2 ($P < 0.003$; Figure 2.4). The total duration spent in the pod was greatest for 3Tea, and the least for -control ($P < 0.001$; Figure 2.5). Likewise, pigs spent the greatest duration with their heads over the bowl in the pods with 3Tea, which was greater than +control, and the least was -control ($P < 0.001$; Table 2.2; Figure 2.5). The pigs performed more NNOB in the pods with -control ($P = 0.002$; Table 2.2, Figure 2.5).

Discussion

The aim of this study was to determine if the pigs possessed a preference for varying varieties of a fermented sorghum-based tea. This would aid in the development of a satiety product for human consumption. Swine are more biologically relevant subjects for flavor preference in hu-mans than a rodent model (Galef & Whiskin, 2003, Brown & Nemes, 2008, Caruso et al., 2014). This is largely due to the decreased phylogenetic distance be-tween humans and swine as opposed to humans and rodents (Walters & Prather, 2013; Fleming et al., 2017; Roura & Fu, 2017; Cirera et al., 2018). However, most research in-volving swine taste preference has been done in young pigs or lactating sows that are ad libitum fed (Clouard & Val-Laillet, 2014; Guzmán-Pino et al., 2014; Wang et al., 2014; Zhu et al., 2016; Reyes-Camacho et al., 2021).

Some swine researchers cite that sorghum is an inferior grain when compared to corn be-cause growing pigs have reduced feed intake and average daily gain when fed a sorghum-based diet (Ronda et al., 2019; Pan et al., 2021). In swine production, the reputation of sorghum may be based off of fermented sorghum offered to pigs is dried distillers' grains, a by-product that could potentially be fed to pigs. Producers are more likely to pick corn based dried distillers grain because of higher levels of metabolizable energy compared to sorghum-based distillers grain (Corassa et al., 2019). Nonetheless, researchers advise that sorghum be properly processed and not be derived from a by-product in order to in-crease bioavailability of nutrients (Brambillasca et al., 2019; McCuistion et al., 2019; Thomas et al., 2020) and the benefits of the antioxidant capacity may im-prove the immune system (Fan et al., 2023) which would be more appropriate for limit fed pigs (De Souza et al., 2023). Therefore, before the health benefits of fermented sorghum tea can be studied in adult pigs, the authors need-ed to determine if the sour and bitter flavors of fermented sorghum tea would be rejected. The hypotheses were that pigs would prefer the tea with the least gallic acid, and then prefer the isocaloric control over the blended fermented sorghum tea.

The isocaloric control was created to contain the same amount of sweetness as the ferment-ed sorghum tea. The culture for this tea formula still requires sugar to activate the SCOBY and then they produce fructose and glucose left over as byproducts (Tran et al., 2022). The formula also produces byproducts that add tartness. Acetic, gallic, glucuronic, and malic acids after fermentation result in the sourness that can cause rejection among subjects that have not been exposed to these flavors (Kittawornrat & Zimmerman, 2011; Bishop et al., 2022). Due to the fermentation process, the fermented tea possesses some carbonation, although humans prefer carbonation (Redondo et al., 2014; Barker et al., 2021; Wendrick et al., 2021). The final layer commonly reported in the flavor breakdown of SCOBY fermented teas is that of bitterness and a slight astringency. This can be attributed higher than normal level of ethanol that is a naturally occurring byproduct of fermentation, but only 1.0% alcohol by volume of ethanol is legally allowed in commercial fermented teas (Jang et al., 2021; Bishop et al., 2022). However, the bitterness and slight astringency is largely attributed to the polyphenol content of the product (Tran et al., 2020; Bishop et al., 2022).

For the current study, during the acclimation and conditioning phase the pigs spent a significant amount of time performing non-nutritive oral behaviors, which in the case of test areas, are interpreted as exploratory behaviors. Pigs first had to adjust to the novelty of the 3-choice maze test arena but reduced exploratory behaviors after each session. Then, they had to learn that at least one of three options contained the positive control (isocaloric water sweetened with sucrose), which is a method used pharmaceutical avoidance or preference research (Nofre et al., 2002).

In the first experiment, three varieties of sorghum tea were evaluated. This first experiment was designed to include four sessions, because the first session would be the first time in the pigs' life that they were exposed to a fermented product. As expected, pigs consumed less tea (regard-less of type) in the first session compared to the three succeeding sessions. Age influences the amount of time pigs spend in an exploratory state (Fleming et al., 2017; Docking et al., 2008). Neophilia increases with age, therefore, in experiment 1, adult pigs displayed less exploratory behaviors and more appetitive behaviors compared to research with young pigs and preference or novelty testing (Fleming et al., 2017; Docking et al., 2008). For the first experiment, the only behavioral measure that was influenced by choice was the non-oral behavior duration measure. Non-oral behaviors could be interpreted as refractory or rather, satiety, behaviors (Jensen, 2017). In appetitive behavioral analyses, animals exhibit three states: first, appetitive (i.e. exploratory), then consumption, which is followed by a refractory period (Urrutia et al., 2021). In a sense consumption of the teas and durations of head-in-bowl were great and similar among all tea types offered, the non-oral behaviors displayed near the white tea bowl indicate either: 1) a level of satiety, or 2) a greater interest in the sumac-based teas. The authors suspect the latter because gallic acid and other polyphenols are associated with satiety in rodent and human trials (Urrutia et al., 2021; Chen et al., 2022), and the white fermented tea in the current experiment had significantly less gallic acid than the sumac teas. The non-oral behaviors associated with the roasted sumac tea were the same as the non-roasted sumac tea, even though gallic acid was reduced by the roasting process (Hithamani & Srinivasan, 2014; de Morais Cardoso et al., 2015). Nonetheless, there are not enough indicators of a preference for a type of tea for the current experiment, but rather adult pigs display high affinity for all three types of teas based on the fact that they consumed nearly all three teas after the first exposure.

The live observation measures (first choice and volume consumed) did not indicate a clear preference during the trials, therefore, in the second experiment, the authors blended the three teas from the first experiment to compare to the positive control (isocaloric water) and negative control (water). For overall consumption, pigs consumed very little of the negative control, while the isocaloric positive control and blended tea were nearly completely consumed. In addition, the greatest duration of exploratory behaviors were observed near the negative control. For preference research, a negative control is needed to determine if both the positive control and substrate of interest will be avoided (Docking et al., 2008; Lipstitch et al., 2010). The refusal volume and exploratory behaviors indicated that the pigs were dissatisfied with the negative control, and avoidance of the blended tea was not observed. Exploratory behaviors include NNOB, which in pigs are also referred to in the literature as oronasal or oral-nasal-facial behaviors because pigs also rub their face and root as a part of NNOB (Hulbert et al., 2019; Studnitz et al., 2007; Tatemoto et al., 2022). These behaviors are commonly studied in sows housed in gestation crates that produce a mentally unstimulating environment. However, the adult pigs in the current experiment were not housed in a mentally stagnant environment and the 3-Choice Maze test added enrichment to their daily activities.

In fact, there were clear indicators that the blended tea was preferred over the isocaloric control, even though they had the same level of calories from sugar. For example, the total amount of time spent in the pod with the blended tea was greater than the positive control and the total amount of time the head was in the bowl were greater the positive control, even though the blend-ed tea had 50-fold increase of gallic acid. As mentioned previously, the acid by-products from fermentation increases the amount of sour, bitter, and astringent mouthfeel (Tran et al., 2020, Bishop et al., 2022, Wang et al., 2022). The results of the current study are in contrast to the assumption that all pigs will reject these added flavors.

Albeit, for the current project, one must note that flavors that pigs have shown to avoid are in addition to the sweet flavors from the SCOBY products (i.e. fructose and glucose; Tran et al., 2022). Figuroa et al. (2019), compared preference-behaviors for umami concentrations (Monosodium glutamate MSG) and compared them to sucrose concentration in young commercial pigs (1 month old). This work demonstrated that although pigs preferred the highest concentration of umami (MSG), they preferred the medium concentrations of sucrose (4-8%; scaled from 0.5-30% solution). This preferred sucrose concentration is comparable to the current project. The positive control solution was 7.2% sucrose, which matched the Brix calculated sugar content of the fermented sorghum teas, thus matching Figuroa et al.'s sucrose findings. The authors of this manuscript can deduce that the added flavors from sorghum and fermentation are preferred. Classic pharmaceutical drug avoidance researchers identified bitter flavors without a sweet foundation. Young adult pigs (2-4 months) that were fasted from food and water for 8-16 hour had thresholds for acceptance of bit-ter compounds when compared to human thresholds [29]. However, more recent researchers identified that water deprivation confounds preference results [40]. In the current work, our pigs were not water deprived, but rather fed at maintenance. Researchers demonstrated in young pigs that they will prioritize palatability over biological value, which is commonly seen in humans as well (Guzmán-Pino et al., 2014; Figueroa et al., 2019). For the current research, the polyphenols have biological value, but the added flavors did not appear to phase the adult pigs. In fact, they prefer the fermented sorghum tea over the isocaloric control, which may be an indication that nutrients may be valued in adult pigs.

There is little published research on adult pig preference on flavors (Guzmán-Pino et al., 2014; Wang et al., 2014). Wang et al., (2014) examined if the supplementation of the feed of 52 Large White lactating sows would im-pact flavor preference in their piglets. The study was conducted to address insufficient feed intake of commercial sows during lactation. There were four treatments consisting of an astringent compound such as sodium butyrate, fruit-milk flavor, and fruit-milk-anise flavor, as well as a control diet. Wang et al., (2014) determined that dams consumed for feed when they were treated with the fruit-milk-anise. Anise is a seasoning that leaves a bitter after taste (Charles, 2012), therefore re-searchers focused on preexposure to flavors via lactation so that piglets have less avoidance behaviors at weaning. The piglets from fruit-milk-anise fed sows had increased in feed intake at weaning, therefore, pre-exposure is key to acceptance of new flavors among young animals. In the current study, the adult pigs had a lack of neophobia for the additional flavors, even though the additional flavors were likely novel. This finding is likely due to the fact that the final product has the foundation of sweetness, but adult pigs that are limit fed are neophilic. Future work will determine if obese pigs have the same affinity to fermented sorghum tea as lean pigs.

Conclusions

The current study showed that adult pigs have a high affinity to fermented sorghum tea, regardless of tea type. Nonetheless, the main limitation of the current study was in experiment 1; preference (or avoidance) among the three tea-types were not determined due to non-significant results. In addition, future work could determine if there is a threshold in sorghum varieties that have very high concentrations of gallic acid, such as black sorghum.

The current work now helps reduce the assumption that all pigs will reject novel, sour and bitter flavors. This research reinforces the legitimacy of the use of swine models for modeling preference in humans and also for improving health in adult pigs. The authors can conclude that the future work exploring the health benefits of fermented sorghum tea will not be impeded by a preference of the isocaloric control over the complex, high polyphenol fermented sorghum tea.

Implications

Sorghum is a highly prolific and sustainable crop that can be grown in many harsh climates, making it a more suitable crop in the face of climate change. Nonetheless, nutritional security and consumer-acceptance play a large role in sustainability; if the products produced from a resilient crop are rejected by consumers, then it cannot be considered a sustainable crop. Therefore, sorghum products must be formulated for acceptance, little waste, and improved health. In this preliminary study, fermented sorghum tea was preferred over an equally sweet positive control among adult pigs that were limit fed to maintain a healthy body weight. If future studies indicate that fermented sorghum tea has health benefits, then the evidence of sorghum as a sustainable crop will be strengthened.

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Tables

Table 2.1. Pig (n = 11) preference behaviors in a 3-Choice maze with fermented sorghum tea type. The choices were fermented tea made from: white, sumac, or roasted sumac (roasted) sorghum. Pigs were ex-posed (Exp) four times after conditioning.

	Choice				Exposure					*p*-Values		
	White	Sumac	Roasted	SEM[1]	1	2	3	4	SEM[1]	Choice	Exp	Choice x Exp
Consumption[2], mL	95.2	97.9	95.6	±2.34	87.9[a]	99.9[b]	99.2[b]	97.9[b]	±2.64	0.628	0.005	0.653
Gallic acid, mg	3.3[a]	13.7[b]	12.7[c]	±0.22	9.1[a]	10.3[b]	10.2[b]	10.1[b]	±0.25	<0.001	0.008	0.201
Duration, %												
Total	28.2	31.8	30.0	±1.25	30.1	29.8	30.5	29.7	±1.48	0.208	0.988	0.922
Bowl[3]	21.9	24.2	23.9	±1.69	23.7	21.0	24.6	24.0	±1.80	0.264	0.213	0.923
Explore[4,5]	3.1	3.3	2.3	±0.92	3.3[a]	4.7[a]	1.7[b]	1.8[b]	±1.01	0.617	0.029	0.958
Non-oral behaviors[6]	3.3[a]	4.3[b,c]	3.8[c]	±0.45	3.1[d]	4.0[e]	4.1[e]	3.9[e]	±0.48	0.050	0.097	0.949
Latency, %												
Bowl[3]	24.4	24.7	26.8	±3.60	30.9	19.3	24.3	26.7	±4.22	0.890	0.327	0.672
Explore[4]	62.7	67.5	74.1	±6.10	56.6[d]	68.7[e]	75.8[e]	71.1[e]	±6.58	0.192	0.060	0.970
Other[6]	23.5	23.8	27.5	±7.85	30.8	19.5	23.4	26.1	±8.10	0.696	0.338	0.626
Rate, no./min												
Bowl[3]	0.68	0.70	0.68	±0.068	0.57[d,e]	0.70[e,f]	0.72[e,f]	0.76[f]	±0.073	0.904	0.069	0.982
Explore[4]	0.40	0.43	0.32	±0.079	0.47	0.36	0.36	0.35	±0.084	0.302	0.360	0.907
Other[6]	0.97	1.15	1.08	±0.120	0.81[a]	1.1[b]	1.2[b]	1.2[b]	±0.126	0.173	0.003	0.962

[1]Largest Standard Error Mean; [2]Total volume consumed (100 mL maximum) data were non-parametric when Choice was considered. The Kruskal-Wallis Test for Consumption Choice was not significant ($\chi^2 = 0.39$; p = 0.82; df = 2) but was significant for gallic acid ($\chi^2 = 97.5$; p < 0.001; df = 2), [3]Head over bowl, head moving, indicating drinking; [4]Exploratory behaviors (i.e. walking or standing without any sniffing, drinking, chomping, licking, rooting, rubbing using the mouth, snout, or face). [5]p-Values were derived from square root transformed data; [6]All non-oral and non-exploratory behaviors; [a,b,c] LS Means p ≤ 0.05; [d,e,f] LS Means p ≥ 0.05 ≤ 0.10.

Table 2.2. Pig (n = 11) preference behaviors in a 3-Choice maze with choices of: distilled water (-control), isocaloric control (+control; water with sugar) or fermented sorghum blended tea (3Tea; equal parts of white, sumac, and roasted sumac). Pigs were exposed (Exp) to the 3-choice test three times.

	Choice				Exposure				*p*-values		
	-control	+control	3Tea	SEM[1]	1	2	3	SEM[1]	Choice	Exp	Choice x Exp
Consumption[2], mL	18.0[a]	96.6[b]	99.0[b]	±2.21	74.1	68.9	70.6	±2.97	<0.001	0.136	0.087
Gallic Acid, mg	0.02[a]	0.20[b]	11.7[c]	±0.03	3.8[a]	3.8[a]	4.3[b]	±0.03	<0.001	<0.001	<0.001
Duration, %											
Total	16.8[a]	34.5[b]	37.7[c]	±1.71	28.4	29.5	31.1	±1.71	<0.001	0.597	0.033
Bowl[3]	5.6[a]	27.6[b]	31.9[c]	±1.87	20.3	21.8	23.0	±1.92	<0.001	0.365	0.091
Explore[4,5]	7.1[a]	3.0[b]	1.4[b]	±1.45	4.4	3.5	3.5	±1.50	0.002	0.866	0.261
Non-oral	4.2	4.1	4.4	±0.45	4.5	4.2	4.0	±0.46	0.583	0.321	0.506
Latency, %											
Bowl[3]	20.8	16.2	14.8	±3.45	13.93	19.59	18.19	±3.61	0.420	0.481	0.681
Explore[4]	60.1[d]	71.2[e]	76.0[e]	±7.55	69.0	65.7	72.6	±7.74	0.086	0.608	0.714
Other[6]	16.2	15.2	13.8	±3.11	12.2	15.6	17.4	±3.26	0.849	0.480	0.958
Rate, no./min											
Bowl[3]	20.75	16.18	14.77	±3.459	0.42	0.48	0.68	±3.615	0.420	0.481	0.681
Explore[4]	0.55[d]	0.38[e]	0.35[e]	±0.108	0.39	0.53	0.37	±0.111	0.102	0.196	0.646
Other[5]	1.41	1.44	1.52	±0.150	1.49	1.46	1.41	±0.152	0.526	0.730	0.413

[1]Largest Standard Error Mean; [2]Total volume consumed (100 mL maximum) data were non-parametric when Choice was considered. The Kruskal-Wallis Test for Choice was significant for consumption volume (χ^2 = 0.71.6; P < 0.001; df = 2), and for gallic acid (χ^2 = 88.9; p < 0.001; df = 2). ; [4]Exploratory behaviors (i.e. walking or standing without any sniffing, drinking, chomping, licking, rooting, rubbing using the mouth, snout, or face). [5]p-Values were derived from square root transformed data; [6]All non-oral and non-exploratory behaviors; [a,b,c] LS Means ≤ 0.05; [d,e] LS Means ≥ 0.05 ≤ 0.10.

Figures

Figure 2.1. Flow chart outlining the procedure for making fermented sorghum tea using symbiotic culture of bacteria and yeast (SCOBY). 500 grams of sorghum was seeped overnight in 1500 mL of distilled water. It was then extracted through a filter and brought to boil where 201 grams of sugar was added and dissolved. 1.9 liters of water was added, and the solution was then cooled to 35° C. The symbiotic culture of bacteria and yeast (SCOBY) was added and allowed to ferment for 7 days at 27° C. Finally, the solution was kept at a refrigerated temperature of 4° C.

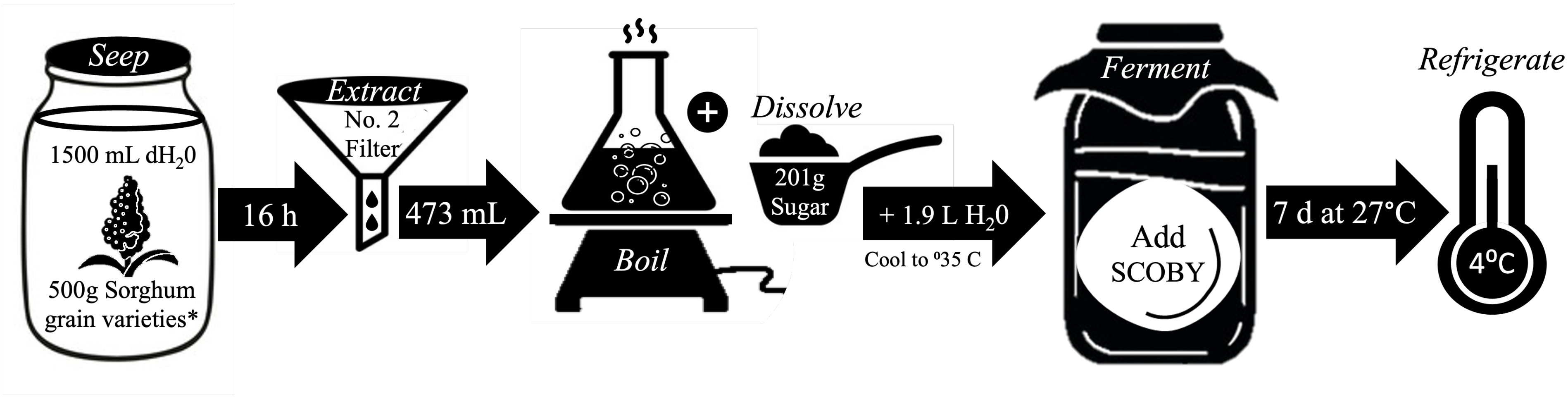


Figure 2.2. 3PodShape Maze diagram- The aisle was used as the chute and two standard gates were used to funnel the pig into the center entrance of the three pods (Entrance). For daily sessions, the subject entered the entrance, a pig board was used (dashed line) to close the maze. A bowl was placed in each pod and 100 mL of solution was offered. Second Panel- Flow chart representing each step of the preference study. [1]The acclimation phase had 100 mL of isocaloric solution (sweet water) in every bowl. After 5 minutes, pigs that did not explore every pod were led into the area with a marshmallow or a feed pellet was dropped into the bowl. [2]The phase did not include leading or extra marshmallows. Three minutes of exploration were allowed. [3]The solutions were randomly assigned to pod so that each pig had a solution placed in at least three different pods.[4]

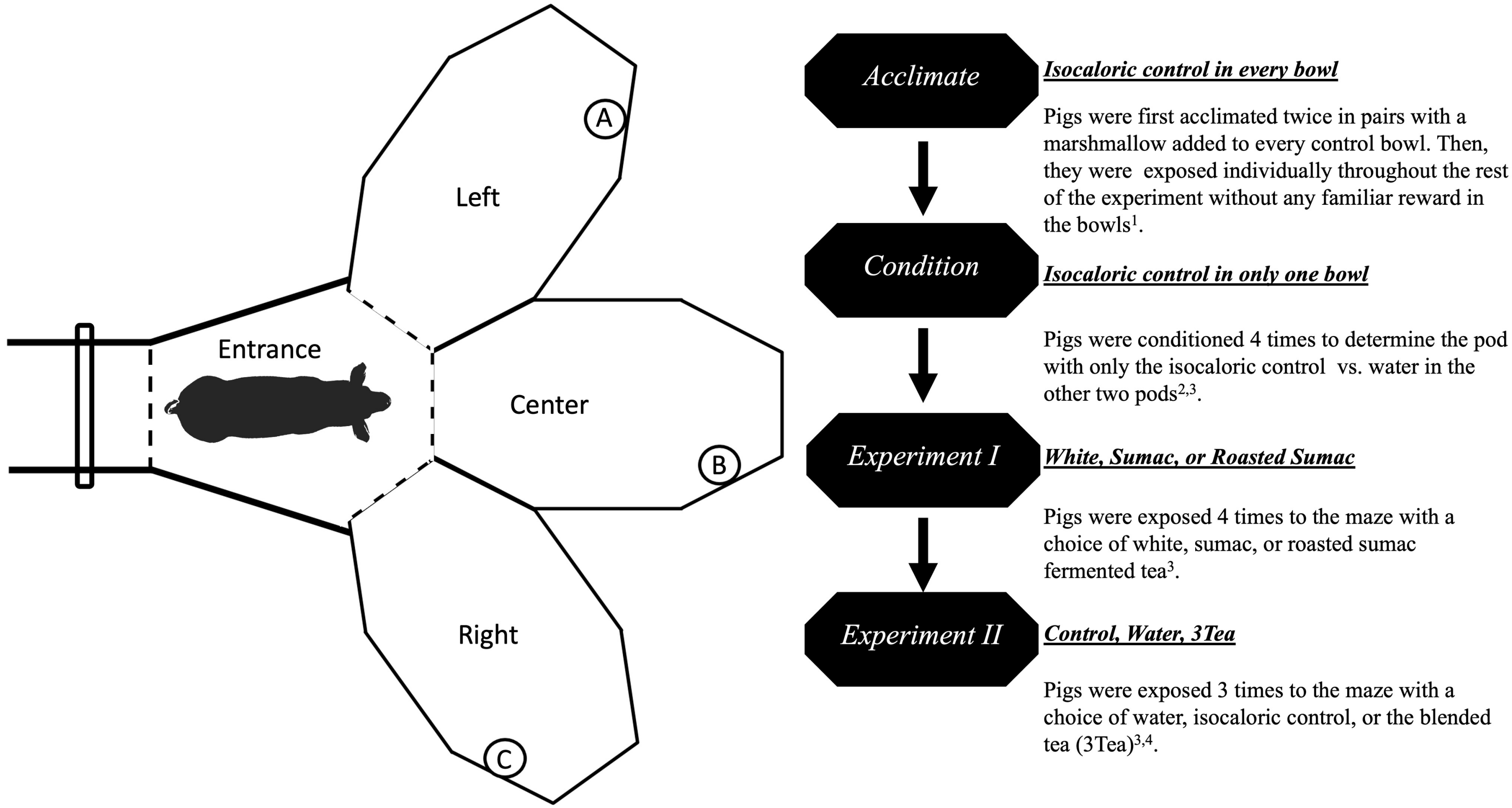


Figure 2.3. Stack Bar Graph of pig (n=11) preference behaviors in a 3-Choice maze with fermented sorghum tea type. The choices were fermented tea made from white, sumac, or roasted sumac (roasted). The entire bar represents the total duration (% of observation) of time spent in pod with the corresponding solution, with the top error bar representing total duration SEM. The bottom stacks rep-resent the duration of head in bowl, the middle stacks represent the percent duration of exploratory behaviors (i.e. non-nutritive oral behaviors), and the top stacks represent the percent duration of all other behaviors (head still, non-oral, walking or standing). [a,b,c]LS Means differed ($p < 0.05$).

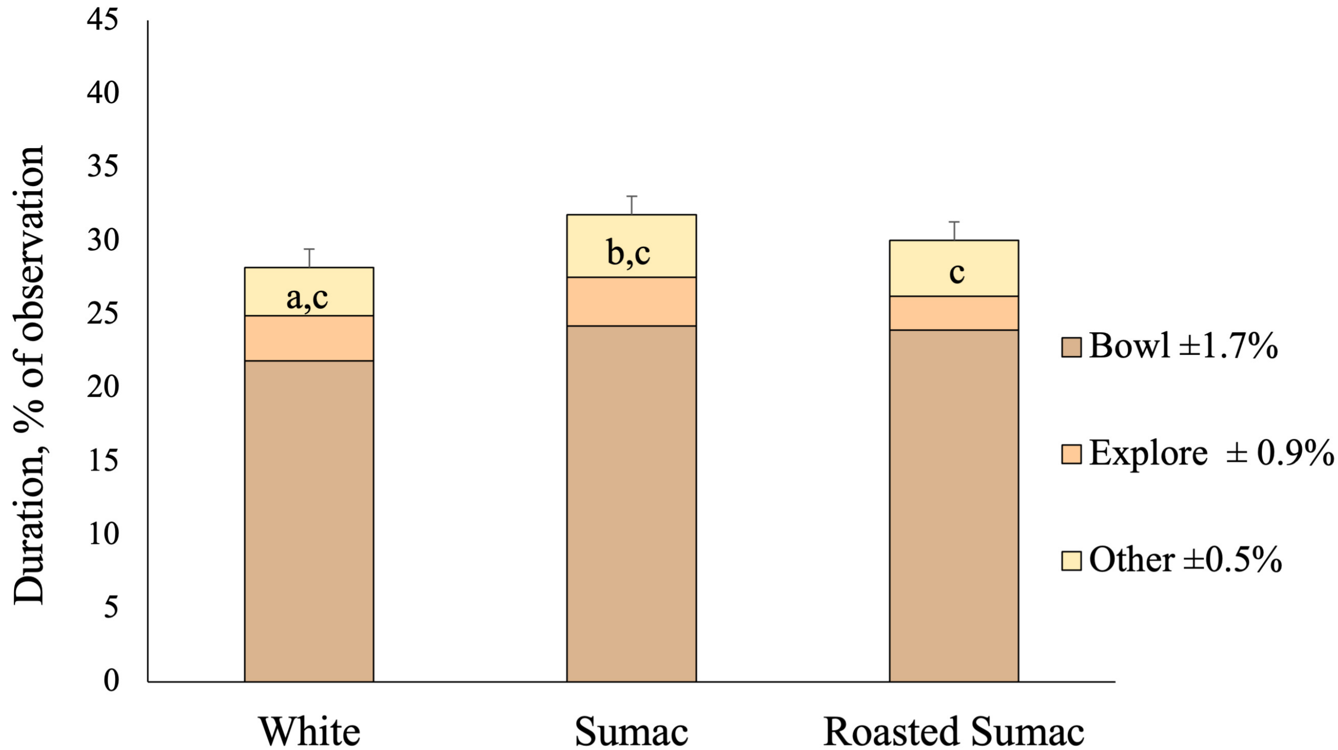


Figure 2.4. Line graph of sessions for total duration pig (n=11) preference behaviors in a 3-Choice maze with fermented sorghum tea type. The choices were: an equal blend of fermented sorghum tea type (3Tea; white, sumac, and roasted sumac); isocaloric solution (-control; sucrose water), or distilled water (+control). The error bars represent SEM. * $p \leq 0.05$ negative control was less than isocaloric control and 3 Tea.

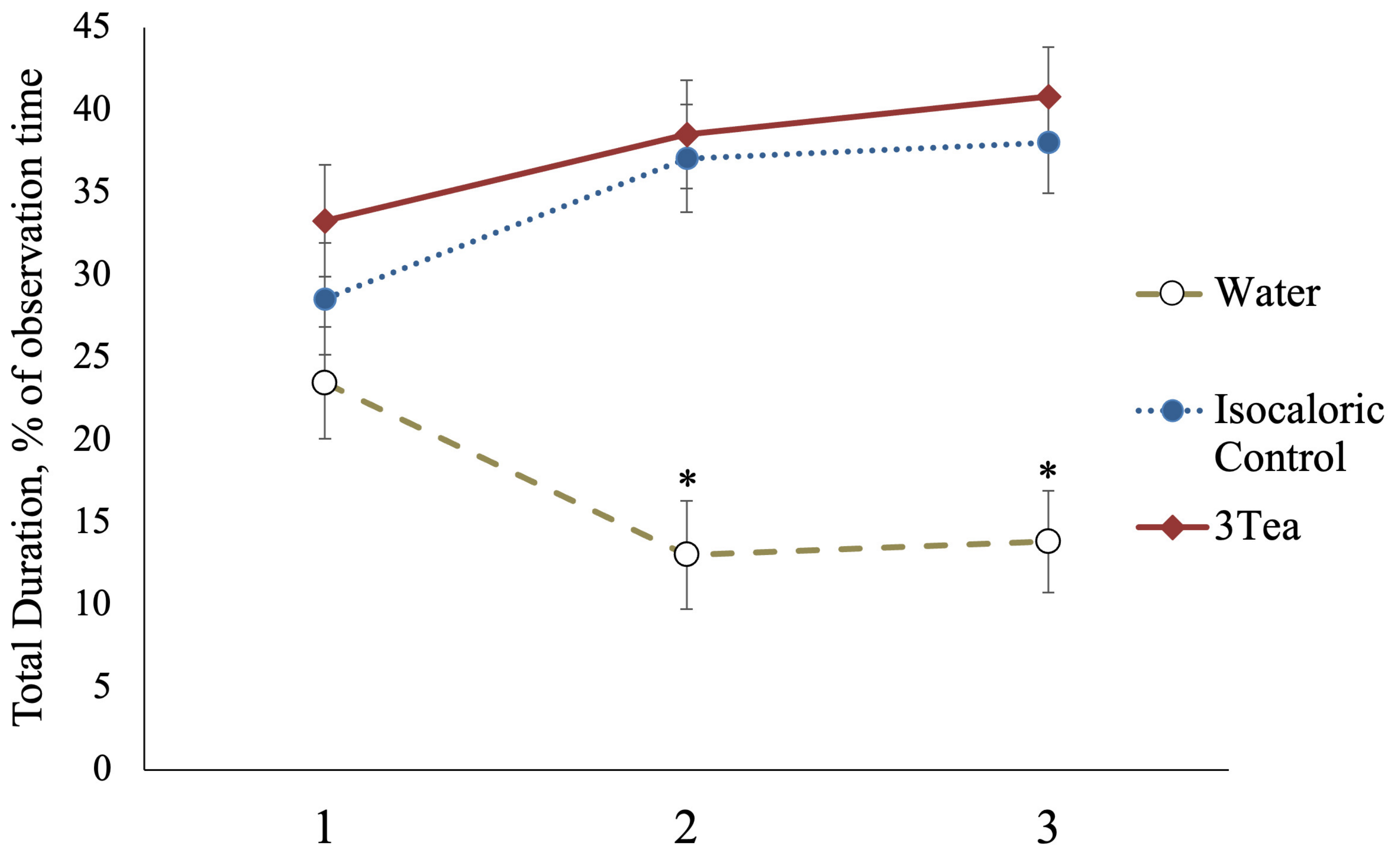
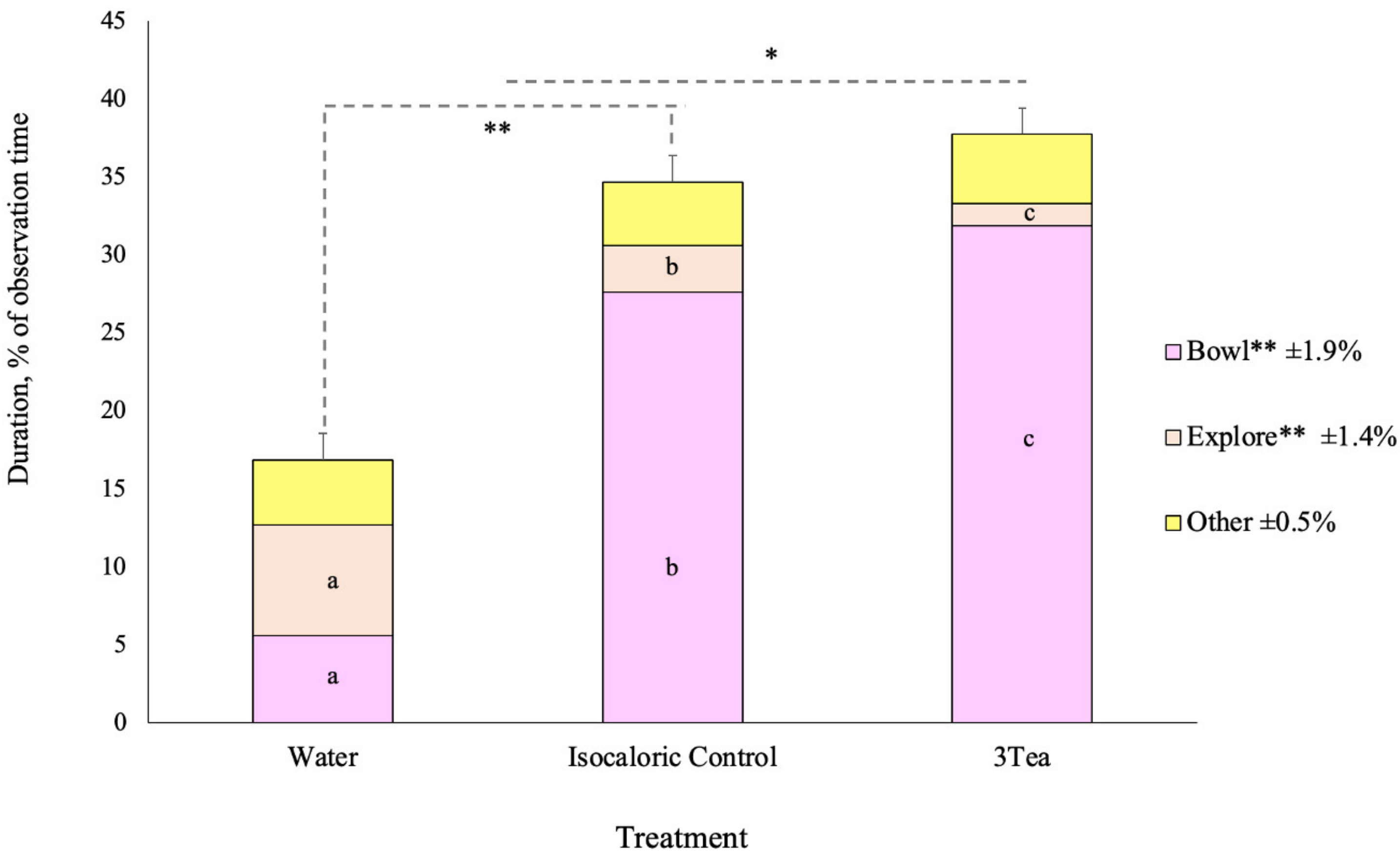


Figure 2.5. Stack Bar Graph of pig (n=11) preference behaviors in a 3-Choice maze with fermented sorghum tea type. The choices were: an equal blend of fermented sorghum tea type (3Tea; white, sumac, and roasted sumac); isocaloric solution (-control; sucrose water), or distilled water (+control). The entire bar represents the total duration (% of observation) of time spent in pod with the corresponding solution, The bottom stacks represent the duration of head in bowl, the middle stacks represent the percent duration of exploratory behaviors (i.e. non-nutritive oral behaviors), and the top stacks represent the percent duration of all other behaviors (head still, non-oral, walking or standing). * $p \leq 0.05$ LS Means for total duration LS means between 3Tea and Isocaloric; **$p \leq 0.01$ for total LS means between –control and the other solutions. [a,b,c] $p \leq 0.05$ LS Means within behavioral category.



Chapter 3 - Social Status Influences Adult Ossabaw Learning and Working Memory During the Development of a Novel Feeding Cognition Test

Abstract

Few practical porcine behavioral tests to evaluate cognition are available to biomedical researchers. Therefore, our objective was to evaluate individual and paired learning using a novel cognition feeder prototype with Ossabaw pigs (n=11; age 15.6 ±0.18 SEM months; barrows and gilts; pair-housed). The first prototype consisted of 4 bowls, with a paddle over each bowl. Pigs were conditioned to press a paddle to trigger a reward (50 g feed). Pigs were conditioned first in pairs, then individually. Following conditioning, they had to distinguish which two paddles and bowls would provide a reward, and each pig had a different format to learn for correct (C) or incorrect (I) paddles (e.g. CICI, ICIC, ICCI, CIIC). Seven sessions were individual testing then three were pair testing. There was no large difference in performance based upon format assigned. Pens assigned the outside format (CIIC) had the highest rate of nose contact with the correct paddle in the first two individual testing stages (*P* =0.002; 3.2 vs. 2.6 vs. 2.6 ±0.42 SEM, no./min) implying a quicker uptake than other formats. The total number of advances and retreats in the testing arena were used to calculate dominance-indices and pigs were categorized as dominant or submissive for each pair. In the conditioning phase, dominant pigs spent more time nosing the paddles than submissives (44.8 vs. 41.9 ±3.52 SEM, %). In contrast, during the individual learning sessions, submissives spent more time nosing the correct paddles than their dominant counterparts (*P* =0.01; 23.5 vs. 35.7 ±2.52 SEM, %). During the trained pair testing phase (Session 12-14), the submissive pigs had the greatest rate of overall paddle contact (*P*

=0.02; 6.2 vs. 7.0 ±0.60 SEM, no./min) as well as the highest rate of contact with the correct paddle (*P* =0.01; 4.3 vs. 4.5 ±0.44 SEM, no./min). A better understanding of behavior in dominant and submissive pigs must be achieved before it can be concluded that social status impacts task performance. The feeder prototype test is intended to become an in-pen feeder for cognition testing among group-housed pigs, however, individual training sessions will be needed to account for social-status learning rates. This work has great potential to evaluate cognition, memory, learning, motivation, and social communication in biomedical pigs, thus improving the translation of preclinical data to clinical outcomes.

Keywords: Swine; Cognition; Ossabaw; Dominance; Porcine; Behavior; Learning; Memory; Contrafreeloading

Introduction

Biomedical researchers are increasing the use of large animal models, such as swine. Pigs have a smaller phylogenetic distance from humans compared to the more commonly used rodent models (Cupitra et al., 2023; Gieling et al., 2011; Lunney et al., 2021; Tang & Mayersohn, 2018; Walters & Prather, 2013). In addition, recent advancements in swine breeding and gene editing capabilities in minipig varieties likely contribute to their increasing popularity in research. Swine cognitive and neurological pathways are very similar to humans, making swine an efficient pre-clinical model for translational research assessing brain functionality and cognition (Conrad et al., 2012; Sauleau et al., 2009; Simchick et al., 2019; Weickenmeier et al., 2018). The sexual dimorphism of swine brain development even mimics that of human males and females (Conrad et al., 2012). Furthermore, the size of the pig brain allows for the utilization of human clinical brain scanners (Kornum & Knudsen, 2011). Nonetheless, the practicality and availability of repeatable and reliable biobehavioral tests for rodent models may impede biomedical researchers from transitioning to a swine model (Denner & Schuurman, 2021; Kenchegowda et al., 2021; Kuzma et al., 2021).

In addition to lack of availability, biobehavioral tests for small animal models are not species specific or appropriate for swine. For example, the Morris water maze can be constructed with a large pool and applied to young, small pigs (Seigford et al., 2008), the pool must grow with the pigs, otherwise the pigs become large enough to use their hind legs and walk upright in the pool. Rodents have an innate fear of water and are highly motivated to swim and search for the platform, whereas pigs are naturally hydrophilic, therefore they may not be motivated to search for the platform.

Some rodent biomedical tests can be species appropriate and scaled up for pigs. For example, hole board test exploits natural behaviors of both rodents and swine to burrow or root (respectively) for the correct answer (Gieling et al., 2013; Antonides et al., 2015). For swine research, this test requires a large testing room with a high ceiling for filming and a large floor space. Therefore, resources or intra-laboratory repeatability is a major drawback to this test. Furthermore, this test requires labor and the removal of pigs from their home environment, which increases the acclimation and conditioning times, which increases feasibility for some neurological models, such as traumatic brain injury (McNeil et al., 2023).

Other tests, such as the human approach test can be conducted in the pigs' home environment (Hulbert et al., 2019; Roelof et al., 2019). The technology for rodent in-pen testing has advanced the ability to both track group housed animals automatically and continuously and apply cognitive tests within the home environment (Kahnau et al., 2023). Therefore, a long-term objective of our research is to develop a similar system for pigs, with focal cognition testing occurring at the feeder. Furthermore, the social interactions of paired or group housed pigs could reveal significant neurobehavioral pathways, but adult pigs in both biomedical research and commercial production systems tend to be single housed, especially for nutritional allocation (Jensen, 2002; McEachern & Schröder, 2002; Fanzo, 2015). Nonetheless, allowing the natural formation of social hierarchy among adult pigs, even at a minimal group (i.e pairs) can allow for more complex neurological behaviors (Wood-Gush et al.,1990; D'Earth & Turner, 2009). In housed in pairs, the dominant and submissive pig could be identified thus, contributing to a better understanding of social cognition (Held et al., 1999). For example, pathologies such as Alzheimer's disease and dementia have prepathological behavioral biomarkers such as social decline (Davis & Sikorskii, 2020). The evaluation of social and behavioral cognition aids in the development of preliminary biomarkers for earlier identification of these deficits. We hypothesized that a novel cognitive feeder system could aid in the development biomedical procedures to assess these factors.

For this current project, a novel feeder prototype was developed to measure the spatial learning and memory capabilities of swine. The ability of the pig to learn this task was assessed through behavioral data collection. Therefore, the objectives of this study were: 1) Test the prototype for a novel in-pen cognition feeding device, 2) Train the pigs to utilize the feeder correctly, 3) Assess if performance is impacted by format type, and 4) Determine if social status influences performance. The authors hypothesize that the pens assigned the outside format would have a stronger performance compared to pens assigned other formats. It was also postulated that the pigs categorized as submissive would be more motivated to complete the test and have a higher rate of successful paddle pushes.

Materials and Methods

Animals and Housing

This study followed the guidelines set forth by the Guide for the Care and Use of Agricultural Animals in Agriculture and Teaching (CorVus IACUC #4745). The animal studies were conducted between May and June 2022. Twelve adult Ossabaw gilts and barrows (age 15.6 ± 0.62 SD months; 53.5 ± 0.62 kg body weight) were pair-housed by sex at indoor, climate-controlled facilities (21°C; CorVus Biomedical, LLC, Crawfordsville, Indiana). Housing conditions and home pen specifications for the pigs are further described in section *2.1* of (Nelson et al., 2023).

Cognitive Testing and Video Recording

A novel feeding system prototype was developed through collaboration between the author's lab and ShapeMaster (CogShape Prototype 1, ShapeMaster, Ogden, IL; Figure 3.1). The feeder prototype consists of four paddles attached to gear operated hoppers that are programmed to release a specified amount of feed (50 g for this study). Upon enrollment, each pair of pigs was randomly assigned an acquisition phase format including inside (ICCI), outside (CIIC), or alternate (CICI; ICIC; Figure 3.2). Between each session, feed was measured and loaded into the hoppers by lifting a lid on the top of each hopper. Once a pig presses an active paddle, feed was released into a fastened 1.9 L bowl. The feeder was connected to a central programmable logic controller (PLC) device. The PLC was used for programming the specific patterns as well as the amount of feed released into the bowl.

A customized network video recorder (NVR) recorded each session continuously at 30 frames per second at a video resolution of 1080 pixels. One researcher was recording real time quantitative and qualitative observations. A single bullet camera (GeoVision Model GV-

EBL4702-2F; GeoVision, Irvine, CA USA) was mounted at 45° towards the testing arena to capture the pigs entering and interacting in the testing arena. The feeder test was started each morning at around 8:30 am. Upon performance of the test, the pigs had not eaten since their PM feeding the day before. There was a total of 100 grams of feed utilized for each feeder test. (15% Crude Protein, 3% Crude Fat; 5% Fiber; DuMOR Hog Grower, Tractor Supply, Brentwood, TN USA). In the earlier phases when all four paddles were active it was divided to equal 25 grams in each hopper. When each pen was assigned a format with only two active paddles, 50 grams was added to both hoppers. Once every pen completed the task, all pigs were given the rest of their ration in their home pens to equal 700 total grams of feed per meal. The feeder, bowls, and pen were cleaned thoroughly in between each test to prevent the presence of olfactory cues.

Working Protocol

The current study was the initial testing of the feeder prototype. A general plan was carried out over 2.5-week period based on Martin and Bateson (2007) flow chart representing the steps involved in behavioral research and testing (Figure 3.3). The strategy was constructed and adapted using the qualitative and quantitative data collected at each session during the first three conditioning sessions.

For all stages of conditioning, every paddle was activated, and every bowl was rewarded. The first goal was to introduce the pigs to the device in pairs, because previous studies involving testing arenas indicated pigs displayed distress from being separated from pen mate(s) and exposed to a novel area (Kanitz et al., 2009; Lucas et al., 2024). The first exposure was considered the novel session; therefore, the goal was to allow both pigs to approach the device and explore without shaping (i.e. human providing cues). The pigs were allowed up to fifteen minutes for this first session, however, if pigs completed eating the reward and started competing for residual feed, the cut off time was five minutes. During the second paired conditioning session, pig behavior indicated that a barrier between two feeders was needed to help pigs focus on their own side of the feeding device. After the goal of introducing the device and adjusting the test arena was met, pigs were conditioned individually. Individual sessions were limited to a maximum of five minutes. If a pig did not press at least one paddle on either side of the paddle, they were shaped. For shaping, the human remained outside of the pen and pointed to a paddle. There was a total of eight instances of shaping within this phase of testing.

Then, the individual acquisition (learning) phase followed conditioning. The randomly assigned formats were applied just to the paddles initially but all four bowls released reward. Shaping was still applied if correct paddle on either side was not pressed after five minutes. Due to the pigs now having strong sense of association between the paddles and feed there was no shaping needed after the first day of acquisition. Once live observation data revealed that pigs consistently pressed correct paddles, the difficulty of acquisition was increased by only rewarding the correct corresponding bowl. Once all pigs were able to press correct paddles without shaping, the cut-off time was reduced to three minutes. The last three sessions consisted of pair testing. This increased the challenge of the test because each pig had to make decisions while competing with a pen mate. Strategies for testing contrafreeloading, cognition and social status were developed through this initial methodology (Figure 3.4).

Video Behavioral Quantification

In order to complete quantitative analysis, videos were retrospectively coded using a specialized time stamping software (Observer 11.3; Noldus Observer, Leesburg, VA, USA). Spatial behaviors were defined immediately after the pig entered the testing arena through the gate and two legs crossed the threshold (dotted line in Figure 3.5A). Behavioral classification for this study included a combination of spatial and structural behaviors. The spatial divisions of the testing arena are as follows: No choice, Hopper A, Hopper B, Hopper C, Hopper D, Around left, Around right, Above left, and Above right (Figure 3.5B). The structural behaviors included: exploratory behaviors (i.e. non-nutritive oral behaviors, NNOB, described previously (Hulbert et al., 2019) in each one of the previously listed spatial zones, eating, and head still. The ethograms for this study also include point events regarding paddle A, B, C, or D releasing feed as the reward. These point events are coded as paddle A, B, C and D (Figure 3.5B). Video playback was done at half speed with a rate of 30 fps. These behaviors were combined to create a mutually exclusive coding scheme. Two trained ethologists coded the same eleven videos and a Cronbach's reliability test was performed using this sample. Mean alphas were 0.87 ± 0.189 SD and 0.87 ± 0.169 SD for duration and frequency, respectively. After the reliability test was performed ethologists divided the videos between individuals and pairs and coded every session. Ethologists were blinded to the correct answer format while coding behaviors.

Dominance Indices Calculation

A dominance indices was applied, then within a pen, the pig with the greater index was classified as dominant, and the other as submissive, using a modified equation (Figure 3 Martin & Bateson, 2007, p.133; Zumpe & Michael, 1986). The equation was modified to account for interactions that only occur between exactly two animals. A matrix was created using frequency advances and retreats for paired sessions. The data were organized in a 2x2 matrix outlined in Martin & Bateson (2007, p.133) to better illustrate the outcome of all the interactions between the two pigs in question. A percentage of aggression was calculated first by taking the number of advances divided by the total number of interactions and multiplied by 100. A submission percentage was then calculated in the same manner by taking the number of retreats divided by the total number of interactions and multiplied by 100. These two numbers are then added together and divided by 2 to get the final dominance index. The pig with the higher percentage was labeled as dominant, while the pig with the lower percentage was labeled as submissive. The expression of dominance as a percentage allows for scaling of all dyadic relationships while still allowing for clear binary categorization.

Statistical Analysis

Statistical analyses were completed using SAS version 9.4 (SAS, Cary, North Carolina/USA, SAS Institute Inc.). A linear mixed model for repeated measures was utilized (Restricted-maximum likelihood; ANOVA). Included were the random variable of pigs and the fixed effects of format (outside, inside, alternate) and social status (dominant, submissive). The repeated measure was session. The covariance for all analyses was compound symmetry. The outcome variables included duration percentage, latency percentage, and rate/number per min. Least-Squares means for all measures was assessed. All data was checked for normality using the Shapiro-Wilkes test in the UNIVARIATE procedure. Data was then Log (10) transformed as necessary. Pairwise comparisons were carried out using the two-sided test; the Tukey-Kramer method was used to adjust for multiple comparisons. Categorical data was assessed using a chi-square method and results are presented as observed, expected and residual. Treatment (Status/Format), time (Session), and Status*Session interactions of $P < 0.05$ were considered significant, and $P \geq 0.05 \leq 0.10$ was treated as a tendency when appropriate.

Results

General Results

During the conditioning phase (Sessions 1-5), one gilt passed away for unknown reasons. She did not complete any testing after the individual conditioning phase and did not receive a status of dominant or submissive. This gilt was also excluded from all statistical data calculations. The goals of testing resulted in 2 paired conditioning session, 3 individual conditioning sessions, 6 individual acquisition sessions, and 3 paired acquisition sessions, with specific modifications made based on live observations (Figure 3.7).

Format

Individual Acquisition (Sessions 6-11)

There were significant Format*Session interactions for the rate of correct paddle contact, with the highest rate occurring during session 6 and correlating with the outside format (*P* =0.002; Table 3.1; Figure 3.8). There was also a significant Format*Session interaction for overall contact with any paddle. The outside format had the highest rate of contact with any paddle which peaked in session 6 (*P* =0.02; Table 3.1; Figure 3.9). Rate of eating had a significant session effect with the highest rate occurring during session 6 (*P* =0.05; Table 3.1). The highest rate of incorrect paddle contact occurred during session 6 (*P* = 0.0004; Table 3.1). This same variable also tended to have a format effect as the lower rate of incorrect paddle contact occurred when the outside format was assigned (*P* =0.06; Table 3.1). The highest rate of total non-nutritive oral behaviors occurred in sessions 6 and 7 (*P* =0.01; Table 3.1). The pigs had a higher rate of visits to the left feeder in session 6 and 7 as well (*P* =0.003; Table 3.1). The highest rate of head-still movement occurred in sessions 6 and 11 (*P* =0.02; Table 3.1).

The pigs spent the longest duration eating during session 10 (*P* =0.0001; Table 3.1). The longest duration of correct paddle contact occurred when assigned the outside format (*P* =0.02; Table 3.1). The longest duration for this same variable occurred during session 11 (*P* =0.03; Table 3.1). The total amount of time spent performing any non-nutritive oral behaviors was highest during session 6 (*P* =0.002; Table 3.1). The pigs had a strong preference for the left feeder early on in testing, with the longest duration of contact being in session 6 (*P* =0.002; Table 3.1).

The longest latency to correct paddle contact took place in session 6 (*P* =0.01; Table 3.1). This variable also had a tendency for a Format*Session interaction with the shortest latency to correct paddle contact occurring when assigned the outside format (*P* =0.08; Table 3.1). The longest latency to perform any non-nutritive oral behavior occurred in session 6 (*P* =0.04; Table 3.1). The longest latency to perform non-nutritive oral behaviors when in contact with any paddle occurred in session 6 (*P* =0.01; Table 3.1). There was a tendency for the highest latency to eat to occur in session 6 (*P* =0.07; Table 3.1). There was another tendency for head still behavior to have a Format*Session interaction with the longest latency to head still occurring in session 9 and when assigned the alternating pattern (*P* =0.06; Table 3.1). The pigs spent the longest duration at the right feeder during session 11 (*P* =0.003; Table 3.1). The pigs had the tendency to have the shortest duration of any paddle contact in session 10 (*P* =0.07; Table 3.1). Finally, there was a tendency for head still behavior to occur the most in session 6 (*P* =0.06; Table 3.1).

Trained Pairs (Sessions 12-14)

There was no significant Format*Session interaction observed for any of the variables for this phase of testing (*P* >0.10; Table 3.2). There was the lowest rate of incorrect paddle contact in session 14 (*P* =0.01; Table 3.2). The pigs performed the greatest rate of total non-nutritive oral behaviors in session 12 (*P* =0.004; Table 3.2). The pigs tended to perform a higher rate of non-nutritive oral behaviors in sessions 12 and 13 when compared to 14 (*P* =0.08; Table 3.2). The pigs spent the most time eating in session 14 (*P* =0.02; Table 3.2). There was a tendency for the longest duration of correct paddle contact to occur during session 13 (*P* =0.07; Table 3.2) and the pigs spent the most time in contact with an incorrect paddle in session 12 (*P* =0.01; Table 3.2). The pigs spent the most combined time performing non-nutritive oral behaviors in session 13 (*P* =0.04; Table 3.2). The pigs had a tendency to spend a longer duration at the right feeder during session 13 (*P* =0.09; Table 3.2). There was a tendency to have a higher rate of incorrect paddle contact when assigned the outside format (*P* =0.08; Table 3.2). Finally, there was another tendency for the shortest latency to enter a head still state occur during session 13 (*P* =0.07; Table 3.2).

Social

Dominance Indices

Pen 14-12 had an index of 80.3% to 19.8% with Adrian (3276) being the dominant pig. Pen 03-06 had an index of 91.4% to 8.6% with La Jefe (3349) being the dominant pig. Pen 03-05 had an index of 79.0% to 21.0% with Memphis (3271) being the more dominant pig. Pen 08-10 had an index of 55.6% to 44.4 % with Padme (3348) being the slightly more dominant pig. Pen 09-07 had an index of 52.7% to 47.3% with Abbott (3281) being slightly more dominant (Table 3.6; Figure 3.10).

Novel Pairs (Session 1), Paired Conditioning (Session 2&3), Individual Conditioning (Session 4&5)

There was a significant Status*Session interaction effect for the performance of non-nutritive oral behaviors in the no-choice zone of the testing arena (*P* =0.02; Table 3.3; Figure 3.11A). The longest duration of this behavior was performed in session 1 (*P* =0.02; Table 3.3; Figure 3.11A). There was another Status*Session interaction effect for the duration of non-nutritive oral behavior in contact with any paddle (*P* =0.05; Table 3.3; Figure 3.11B). The longest duration of paddle contact occurred in session 5 (*P* =0.05; Table 3.3; Figure 3.11, B).

The pigs had a higher rate of contact with any paddle during the last paired conditioning session (Session 3; *P* =0.003; Table 3.3). There was a higher rate of head still behavior being performed in the paired conditioning sessions (Sessions 2&3) while the lowest rate occurred in the last individual conditioning session (Session 5; *P* =0.0001; Table 3.3). The highest rate of non-nutritive oral behaviors occurring in the no choice zone was in the first paired conditioning session (Session 2; *P* <0.0001; Table 3.3).

The pigs spent the most combined time performing non-nutritive oral behavior in session 2 (*P* =0.04; Table 3.3). The pigs spent more time performing non-nutritive oral behaviors at both right and left feeders in the individual conditioning phases (Sessions 4&5; *P* =0.01; Table 3.3). There was also a consistent decrease over all five sessions of amount of time the pigs spent performing head still behavior (*P* <0.0001; Table 3.3). The pigs had the longest latency to eat in session 1 (*P* <0.0001; Table 3.3). The longest latency to perform a head still behavior occurred in session 5 (*P* =0.05; Table 3.3). The pigs also had the longest latency to perform non-nutritive oral behaviors in the no choice zone in session 4 and 5 (*P* < 0.0001; Table 3.3).

Individual Acquisition (Sessions 6-11)

There were no Status*Session interactions observed for any of the variables for this phase of testing (*P* >0.10; Table 3.4). The smallest rate of contact with both the correct and incorrect paddle occurred in session 10 (*P* =0.01, *P* =0.001; Table 3.4). The lowest combined rate of all non-nutritive oral behaviors was performed in session 10 as well (*P* =0.001; Table 3.4). Session 10 also saw the lowest rate of overall contact with any paddle (*P* =0.001; Table 3.4). Dominant pigs also performed more non-nutritive oral behaviors in the no-choice zone (*P*=0.04; Table 3.4). Session 11 saw the highest rate of head still behavior (*P* =0.02; Table 3.4).

Pigs spent the most time eating in session 10 (*P* =0.0003; Table 3.4). Submissive pigs had a longer duration of contact with the correct paddles (*P* =0.01; Table 3.4). Submissive pigs also had a longer duration of contact with any paddle when compared with the dominant pigs (*P* =0.05; Table 3.4). Sessions 6 and 11 also saw the longest combined duration of non-nutritive oral behaviors performed (*P* =0.01; Table 3.4). Session 10 saw the lowest durations for both head still and non-nutritive oral behavior variables performed around the feeders (*P* =0.03; Table 3.4). Pigs spent time performing non-nutritive oral behaviors at the left feeder in session 6 (*P* =0.04; Table 3.4). Concurrently, the right feeder saw the longest duration of contact in session 11 (*P* =0.003; Table 3.4).

The longest latency to eat was in session 6 (*P* =0.02; Table 3.4). Pigs had the greatest latency to press the correct paddle in session 6 (*P* =0.003; Table 3.4). Pigs had the longest latency to perform any non-nutritive oral behavior in session 6 (*P* =0.01; Table 3.4). The longest latency to press any paddle was in session 6 (P =0.01; Table 3.4). There was a tendency for all pigs to have a longer duration of contact with the incorrect paddles in sessions 6 and 11 (*P* =0.06; Table 3.4). There was also a tendency for individual pigs to have the longest combined latency to perform any non-nutritive oral behavior in session 6 (*P* =0.07; Table 3.4). This same variable also had a tendency for dominant pigs to have the longer latency to perform any non-nutritive oral behavior (*P* =0.07; Table 3.4). Dominant pigs had a tendency for a higher rate of visits to the left feeder in the earlier sessions (*P* =0.06; Table 3.4). Pertaining to this same variable, dominant pigs also had the tendency to have a longer latency pressing any paddle when compared to submissive pigs (*P* =0.08; Table 3.4).

Trained Pairs (Sessions 12-14)

There were several significant Status*Session interactions during this phase of testing. Submissive pigs had a greater rate of contact with the correct paddle, especially in session 13 (*P* =0.01; Table 3.5; Figure 3.12). Submissive pigs also had a higher rate of contact with any paddle (*P* =0.02; Table 3.5; Figure 3.13). There were significant Status*Session interaction effects for the rate of contact with the right and left feeders (*P* =0.04; Table 3.5; Figure 3.14). Dominant pigs performed a higher combined rate of non-nutritive oral behavior in any zone, which showed a drop-in session 14 to create a Status*Session interaction effect (*P* =0.004; Table 3.5; Figure 3.15A). Dominant pigs also spent a longer time performing non-nutritive oral behaviors around either feeder, with a drop-in session 14 creating another significant Status*Session interaction effect (*P* =0.03; Table 3.5; Figure 3.15B).

There was a lower rate of contact with incorrect paddles in session 14 (*P* =0.03; Table 3.5). Session 14 saw a significant decrease in the rate of non-nutritive oral behaviors performed around either feeder (*P* =0.03; Table 3.5). Submissive pigs showed tendencies to perform a higher rate of head still behavior as well as non-nutritive oral behavior in the no choice zone (*P* =0.07; Table 3.5). Pigs spent a longer duration eating in session 14 (*P* =0.02; Table 3.5). The pigs spent the longest duration in contact with the correct paddle in session 13 (*P* =0.04; Table 3.5). In contrast, the pigs had the longest duration of contact with any incorrect paddles in session 12 (*P* =0.03; Table 3.5). The pigs spent the most combined time performing any non-nutritive oral behaviors in session 13 (*P* =0.01; Table 3.5). There was a tendency to have the highest rate of eating occur in session 12 (*P* =0.06; Table 3.5).

Dominant pigs had a longer latency to pressing a correct paddle (*P* =0.02; Table 3.5). Dominant pigs also had a greater latency to pressing any paddle (*P* =0.05; Table 3.5). There was also a tendency for dominant pigs to have a longer latency to perform any exploratory non-nutritive oral behaviors compared to submissive pigs (*P* =0.07; Table 3.5). This same variable also showed a tendency for a shorter latency to perform any non-nutritive oral behaviors in session 13 (*P* =0.08; Table 3.5). There was another tendency for the pigs to have a shorter latency to perform head still behaviors in session 13 (*P* =0.06; Table 3.5). They also possessed a longer latency to perform non-nutritive oral behaviors in the no choice zone (*P* =0.05; Table 3.5). This same variable tended to be the longest in session 14 (*P* =0.08; Table 3.5).

Recommendations for Testing Protocol

Based upon the results found within this study a new protocol can be refined to utilize this prototype more effectively and efficiently. The current pilot study was also completed over a short period of time (~17 days; Figure 3.7). This procedure can be adapted for longer term studies. As this was the first time that the feeder was being used, testing decisions and phase allocation was being determined as the experiment was being performed. When thinking about how to allocate time going forward a primary goal for testing must be determined. In order to assess true contrafreeloading behaviors, a greater number of paddle presses can be programmed while also tracking all paddle presses (Figure 3.4). This will further offer the opportunity to evaluate which pigs are displaying greater cognitive effort through completion of the task and which pigs would rather adapt an alternate strategy such as stealing or freeloading. The number of paddle presses required to trigger the food reward was set to one for the entirety of this study, however, this can be increased to raise the difficulty of the test for the pigs. Pigs that are considered contrafreeloaders will likely record an even higher number of paddle presses while non-paddle pressers may decide to steal food or wait for their partner to move away from the rewarded bowl. In this experiment, a greater proportion of non-paddle pressers were dominant pigs, therefore, these pigs are more likely to use force. A small portion of incidences of non-paddle pressing was among submissive pigs, as these pigs are likely to wait for the opportunity of their pen mate to move away (Table 3.7).

For the current experiment, only the learning phase (acquisition) was tested. Although pairing animals after individual training adds a component of working memory, reversal phases would allow for the comparison to classic mazes (e.g. 8 radial maze). A reversal period is often used in spatial learning and memory tasks to assess cognitive flexibility when the format is reversed (Inside ⇔ Outside; A, Alternating ⇔ B, Alternating; Dilger & Johnson, 2010; Kornum & Knudsen, 2011; Bernheim et al., 2016; Figure 3.4). The authors recommend that the reversal phase follow the same format as acquisition, where the subjects are allowed to make decisions without social pressure first. This feeding test also allowed for the ranking of social status. In pigs, the 'one hole feeder test' was a method for determining dominance vs submission (McGlone et al., 1987; Rudine et al., 2007). That test requires a modification of the daily feeding routine, whereas this new feeding test potentially quantifies advances and retreatments over time, allowing the researchers to track the change in the relationship (Figure 3.4; Figure 3.6B). The authors determined that individual testing sessions were essential to fully assess the pig's ability to learn without the potential interruption of a pen mate. As a result, the formats were first introduced within this phase of testing to allow for the pigs to be cognitively tested without the influence of their pen mate. However, it is important that testing begins with group sessions as neophobia can confound results in prey animals such as pigs (Figueroa et al., 2013). Due to this possibilty, it is important to introduced pigs to the testing arena in pairs if feasible. However, paired sessions are still essential as a lack of social facilitation and competition can limit consumption and feeding behaviors on a reward-based task (Vargas et al., 1987; Bus et al., 2021).

Discussion

Paddle Format

Formats did not have a strong impact on overall performance. Once formats were assigned in session 6, the pigs assigned the outside format had the highest rate of contact with the correct paddle in the first two sessions, displaying a faster understanding of this format. The authors postulate that this is due to how the outside format most closely resembled the distance in which the pigs consumed their normal meals in their home pen. As these pigs were not used to eating right next to each other, this could be an explanation as to why the pigs that were assigned the outside format adapted quicker than pigs with other formats. This is not likely to be an issue moving forward as this feeder will be transitioned to an in-pen method of testing. However, the pigs still exhibited a strong ability to uptake the feeder task with any format as there was consistent paddle contact for all formats throughout the individual learning phase. This was expected since pigs have a strong spatial memory and a high propensity for learning (Bolhuis et al., 2013).

Social Cognition

For the current study, dominant and submissive pigs showed differing strategies when completing the feeding task. Presumably due to their increased size, dominant pigs adapted a displacement strategy to obtain feed. This is likely due to their higher likelihood of success in agonistic interactions (Otten et al., 2002; Bolhuis et al., 2005). Submissive pigs showed a greater motivation for obtaining feed through cognitive completion of the feeder task. This was shown through having a higher rate of paddle pushes overall and a higher rate of correct paddle pushes. The ability of the pigs to adapt these differing strategies has the potential to be attributed to the concept of "perspective taking." Originating in social psychology, this theory outlines the ability to not only process a situation from the perspective of another party but recognize that those thoughts likely differ from your own (Johnson, 1975; Gehlbach, 2023). This concept has been studied with pigs in competitive feeding situations, where submissive, naïve pigs could exploit the information by simply observing the dominant trained pigs to locate the correct reward (Held et al. 1999). The ability to observe and gather information is indicative of higher situational processing including deception, self-awareness, and overall theory of the mind (Towner, 2010; Marino and Colvin, 2015).

All pigs showed a strong uptake in the feeder task as contact with paddles significantly increased from session one to five. There was an extinction of non-nutritive oral behaviors in the individual conditioning sessions (Sessions 4&5). This coincided with an increase in contact with any paddle in the same sessions. Submissive pigs started out with lower paddle contact in session one but surpassed the dominant pigs in once allowed to run the test without the presence of their dominant pen mate in sessions four and five. Submissive pigs continued this trend into individual testing as they had a significantly longer duration of correct paddle contact. Once reunited for the trained pairs phase of testing the dominant pigs had a higher rate of correct paddle contact in the first run (Session 12). Over the course of three sessions dominant pigs experienced a significant decrease in correct paddle contact while being surpassed by the submissive pigs. This pattern was reflected as well in rate of contact with any paddle. The submissive pigs displayed a more confident affective state through a decrease in submissive behavior and an increase in test performance (Boissy et al., 2007; Rutherford et al., 2012). This confidence was likely gained through the opportunity to perform the test individually.

Testing strategy can also be impacted by whomever the pig is with when completing the task (Held et al., 2010). Given the opportunity to perform the test alone, submissive pigs were able to build a stronger performance that could be applied in the later paired sessions. This is why individual learning sessions are essential as it may lead to more cooperative interactions and thus, more stable relationships. Stable groups for pigs are necessary to mitigate undesired behaviors such as agonistic interactions, mounting, tail biting, and nose bellying (Desire et al., 2015; Goumon et al., 2020). However, in swine breeding operations, there can be considerable re-mixing or commingling of pigs, especially once sows return to the gestation barn. A more robust version of the current feeding test may allow producers to track social harmony in groups of sows.

This novel feeding devices also has the potential for preference testing (Goursot et al., 2018; Leliveld, 2019). Laterality has been assessed in pigs using the approach-withdrawal hypothesis that right biased pigs display more dominant behaviors (Goursot et al., 2019). The current study was not designed to determine laterality; a greater number of animals and repetition of the test would be needed. Future work with this feeding device may help determine if the null hypothesis of laterality and dominance can be accepted.

Contrafreeloading

The strategies adapted by the pigs can be further classified as freeloading and contrafreeloading. The latter has been studied in multiple species with most preferring an effort-based reward over a readily available one (De Jonge et al., 2008; McGowan et al., 2010; da Silva Vasconcellos et al., 2012; Schepisi et al., 2016; Rosenberger et al., 2020; Smith et al., 2021; van

Zeeland et al., 2023). The former being interpreted as the prioritization of cognitive task competition to gain more feed while the latter focuses on social dominance to achieve the same goal. While feed acquisition remains as a primary form of motivation for the feeder test, it is postulated that the differing approaches to task completion could impact dopamine signaling and release (Anselme, 2023). As dopamine is known for being essential to motivational behaviors it is likely that its concentration and activity would produce a behavioral impact (Wise, 2004; Salamone et al., 2016). There was a significant correlation between submissive status and contrafreeloading as well as dominant pigs being freeloaders. This was classified based upon the submissive group of pigs having a much higher number of first paddle presses than the dominant group of pigs. Thus, demonstrating a greater amount of cognitive effort. The strategy of becoming a contrafreeloader was likely adapted by the submissive pigs as an attempt to still obtain a large of a portion of the reward as possible despite their physical disadvantage. The dominant pigs were not motivated to complete the test when in their pairs as it was easier for them to displace the submissive pigs and take over that bowl. This can also be seen in individual sessions as the dominant pigs had a significantly lower duration of contact with the correct paddle. Humans and animals alike are prone to take the path of least resistance, this is detailed fully within the concept of game theory (Bshary & Oliveira, 2015; Avila & Mullon, 2023). The dominant pig likely finds it easier to assert physical dominance to meet their needs while the submissive pigs will allocate their effort to a cognitive strategy to acquire feed. Both strategies require some degree of effort to reach the same goal.

Dopamine and Motivation

Pigs are a cognitively complex animal, and the benefit of cognitive enrichment is an alternative drive behind the behavior and performance during the feeding test. Prior literature has established that pigs have a lower welfare state when housed in barren environments (Beattie et al., 2000; Luo et al., 2020; Ludwiczak et al., 2021). Pigs in enriched environments have stronger reference and working memories (Grimberg-Henrici et al., 2015). Working memeory being an essential component of this test. Animals housed in enriched environments also show higher levels of extracellular dopamine (Zentell, 2021). Enrichment creates an improved neurochemical state for learning as D1 and D5 receptors enhance early long-term potentiation (LTP; Otmakhova & Lisman, 1996). The process of LTP involves the persistent strengthening of the synaptic pathways. The result is a long-term strengthening of synaptic communication that is the basis for synaptic plasticity and hippocampal memory (Bliss & Collingridge, 1993; Lynch, 2004). Swine are utilizing complex neurological processes to complete this task. Activation could likely be observed in the pre-frontal cortex (Executive functioning), hippocampus (spatial memory recall), as well as the insular cortex (social decision making) are just a few of the major structures the pigs are utilizing during the feeding test (Kornum & Knudsen, 2011; Bolhuis et al., 2013; Rogers-Carter & Christianson, 2019). The expression of the dopaminergic reward cycle could be secondarily responsible for the strategies adapted by dominant and submissive pigs. As the cognitive challenge the feeder task provides offering an enriching benefit to the pigs that work to complete it. The cognitive complexity that swine are capable of is a large part of what makes them such a viable subject for brain based translational research specifically.

Motivation was likely primarily food based as there was no other source of freely available feed as seen in other studies (Lunney et al., 2021; Tang & Mayersohn, 2018; Walters & Prather, 2013). However, there is the potential for neurochemical differentiation between freeloaders (dominant pigs) and contrafreeloaders (submissive pigs) that could have secondary motivational affects impacting test performance. Pigs undergoing a test involving predictive reward delivery are already experiencing dopamine release within areas such as the ventral and dorsal striatum (Anselme, 2023). This occurs as appetitive motivational behaviors are related to increases in mesolimbic dopamine concentration and greater catecholamine distribution from the nucleus accumbens (NAc; Arias-Carrión et al., 2010; Salamone & Correa, 2012; Salamone et al., 2016). Dopamine supplementation shows increased contrafreeloading behaviors such as excessive checking to a compulsive level which further supports the submissive pig behaviors being indicative of contrafreeloading status as well as increased dopamine activity (Frederick & Cocuzzo, 2017). The possible correlation between social status and plasma catecholamine levels is not well understood. Future studies should focus on neurotransmitter collection of dominant and submissive pigs in order to validate this hypothesis.

Improved cognition, learning and memory assessment has the potential to become a more standardized early biomarker for decreased affective state, and by extension improve animal welfare (Mendl & Paul, 2004; Boissy et al., 2007; Brydges & Braithwaite, 2008). Cognitive performance tests will act as the bridge between mental and physiological wellbeing. This puzzle piece in combination with the understanding of how social status influences performance on cognitive tests such as this one will change the way this data is collected and interpreted in the future.

Conclusions

The current study showed that all the pigs had a strong performance on the feeder test. Dominant pigs showed a lack of motivation over the course of paired testing. Submissive pigs, however, exhibited more initiative for actual task completion as seen mainly through a higher rate and duration of contact with the correct paddles. The feeder prototype will provide an efficient and novel option for in-pen cognitive testing. Further experimentation utilizing the feeder could lead to the discovery of neurochemical differentiation between dominant and submissive pigs that would impact cognitive performance while also highlighting the importance of cognitive enrichment to the neurological wellbeing of the pig. The current work displays the impact of sociality on performance; hence, individual testing sessions are essential to determine the learning ability of the pigs in the absence of social pressure. This was a foundational study utilizing a small population of pigs over a very short period. Further work is needed to validate and develop these techniques as well as to truly determine experimental differences between social status and format. Future work could implicate the use of lean and obese models to assess the impact of metabolic syndrome on cognitive and social pathways.

Implications

The feeder prototype has already been improved based upon the findings of this study. Visual and audio cues have been added to address a dispensing delay that was noticed upon testing. The feeder was also constructed into a booth form with a solid divider in the middle to better differentiate between the two sides. Consistent and comprehensive cognitive collection methods are essential to improving the scientific communities' ability to understand and measure animal behavior. The feeder can also provide mental stimulation in otherwise barren environments, thus improving the welfare of pigs in both production and biomedical scenarios. In this preliminary study, the pigs strongly uptake the feeder task regardless of format, and submissive pigs showed higher motivation to press paddles and trigger the reward. Further studies with larger sample sizes are necessary to validate the hypothesis that dominant pigs tend to be freeloaders while submissive pigs are adapting the contrafreeloading strategy. This aspect of swine sociality is important to understand for the full implications of this research to be realized.

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Tables

Table 3.1. Pig (n=11) performance behaviors during the individual learning phase of testing (Session 6-11). The formats consist of outside (CIIC), Inside (ICCI), and alternate (CICI, ICIC).

	Format				Session							*P*-Values		
	Outside	Inside	Alternate	SEM[1]	6	7	8	9	10	11	SEM[1]	Format	Session	Format x Session
Rate, no./min														
Eat[3]	0.68	0.60	0.61	±0.05	0.79	0.57	0.69	0.54	0.61	0.59	±0.06	0.42	0.08	0.72
NNOB Correct Pad[4]	3.2	2.6	2.6	±0.42	3.3^{a}	3.0^{ab}	3.1^{a}	2.3^{ab}	1.8^{b}	3.1^{a}	±0.29	0.55	0.0002	0.002
NNOB Incorrect Pad[5]	2.3	1.6	1.6	±0.25	2.6^{a}	2.4^{ac}	2.0^{abc}	1.2^{bc}	1.2^{b}	1.7^{abc}	±0.26	0.06	0.0004	0.35
Total NNOB[6]	17.4	21.7	21.5	±3.15	25.2^{a}	25.2^{a}	19.0^{a}	19.2^{b}	12.2^{b}	20.3^{ab}	±2.82	0.52	0.01	0.47
NNOB Paddle[7]	5.5	4.2	4.3	±0.59	6.0^{a}	5.6^{a}	5.2^{ac}	3.6^{bc}	3.0^{b}	4.8^{ac}	±0.46	0.19	<0.0001	0.02
NNOB Around[8]	2.0	4.9	4.8	±1.64	5.5	5.2	3.2	3.7	1.9	3.8	±1.16	0.33	0.12	0.54
NNOB Left[9]	3.50	4.4	4.1	±0.71	6.1^{a}	5.4^{a}	3.6^{ab}	3.4^{ab}	2.1^{b}	3.4^{ab}	±0.72	0.62	0.003	0.59
NNOB Right[10]	3.3	3.7	4.0	±1.15	4.0	4.2	4.1	2.9	2.4	4.2	±0.79	0.88	0.24	0.73
Head Still[11]	2.2	2.1	1.8	±0.21	2.4^{a}	2.0^{a}	2.1^{a}	1.6^{a}	1.6^{a}	2.4^{a}	±0.22	0.23	0.02	0.20
No Choice NNOB[12]	0.0	0.1	0.1	±0.07	0.10	0.1	0.1	0.01	0.03	0.04	±0.05	0.53	0.72	0.45
Latency, %														
Eat[2,3]	9.8	9.6	7.7	±1.15	14.6	8.2	7.4	8.2	8.8	8.3	±1.72	0.35	0.16	0.59
NNOB Correct Pad[4]	2.5	3.2	2.2	±0.79	4.2^{a}	2.8^{ab}	3.3^{ab}	1.7^{b}	1.7^{b}	1.9^{b}	±0.60	0.43	0.01	0.06
NNOB Incorrect Pad[5]	9.4	12.1	16.7	±5.48	8.5	6.7	12.6	14.6	14.2	19.9	±7.04	0.50	0.82	0.71
Total NNOB[6]	2.0	1.8	1.7	±0.30	2.5^{a}	1.7^{ab}	2.2^{ab}	1.2^{b}	1.6^{ab}	1.9^{ab}	±0.30	0.73	0.04	0.28
NNOB Paddle[7]	2.0	2.6	1.9	±0.46	3.1^{a}	1.7^{b}	2.3^{ab}	1.2^{b}	1.6^{b}	1.9^{ab}	±0.38	0.90	0.005	0.06
NNOB Around[8]	45.8	37.5	34.0	±12.92	23.5	35.8	39.8	36.9	56.6	42.0	±11.58	0.86	0.22	0.15
NNOB Left[2,9]	2.2	4.0	2.6	±0.44	3.0	3.1	2.7	1.8	2.5	4.4	±1.14	0.05	0.79	0.96
NNOB Right[10]	17.2	10.1	11.8	±5.50	15.5	8.1	19.1	13.4	13.5	8.5	±4.31	0.59	0.32	0.13
Head Still[11]	14.4	14.2	15.2	±4.61	15.9	8.9	16.8	21.0	13.8	11.1	±4.28	0.97	0.37	0.06
No Choice NNOB[12]	100.0	87.1	90.5	±9.68	82.0	89.5	90.9	98.5	95.8	98.5	±8.18	0.57	0.61	0.91
Duration, %														
Eat[3]	36.2	41.6	48.1	±5.20	28.8^{a}	36.7^{a}	42.5^{a}	46.9^{b}	59.2^{b}	37.6^{ac}	±4.32	0.17	0.0001	0.45
NNOB Correct Pad[4]	35.7	30.0	26.6	±5.62	35.3	32.4	34.6	28.7	22.3	31.4	±4.45	0.42	0.23	0.11
NNOB Incorrect Pad[5]	11.9^{a}	7.7^{b}	7.2^{b}	±1.150	10.3^{a}	11.03	7.4^{a}	7.7^{a}	5.9^{a}	11.2^{a}	±1.32	0.02	0.03	0.23
Total NNOB[6]	51.1	46.4	42.6	±4.03	56.7^{a}	53.3^{a}	47.3^{a}	41.5^{a}	31.8^{b}	49.5^{a}	±4.24	0.24	0.002	0.45
NNOB Paddle[7]	47.7	37.8	33.9	±4.91	45.9	43.5	42.0	36.4	28.3	42.6	±4.52	0.12	0.07	0.19
NNOB Around[8]	3.4	8.0	7.4	±8.04	9.1	8.3	4.9	4.9	3.4	6.6	±2.10	0.48	0.11	0.17
NNOB Left[9]	24.6	25.0	21.3	±4.42	35.3^{a}	26.1^{a}	19.3^{b}	22.3^{b}	18.5^{b}	20.3^{b}	±3.43	0.65	0.002	0.09
NNOB Right[10]	26.5	20.8	21.04	±3.0	20.7^{ab}	26.5^{a}	27.8^{a}	19.2^{a}	13.3^{b}	29.2^{a}	±3.02	0.30	0.004	0.35
Head Still[11]	8.8	11.1	7.3	±3.16	12.2	8.1	8.1	7.8	7.3	10.9	±1.88	0.47	0.06	0.21
No Choice NNOB[12]	0.0	0.6	0.6	±0.70	0.7	0.8	0.4	0.2	0.1	0.2	±0.53	0.76	0.88	0.65

[1]Largest Standard Error Mean; [2]Non-parametric data that became normal upon log transformation. Data was then transformed back. [3]Head over bowl and consuming feed after reward is dispensed. [4]Non-nutritive oral behaviors associated with the correct paddle (i.e. walking or standing without any sniffing, drinking, chomping, licking, rooting, rubbing using the mouth, snout, or face). [5]NNOB associated with the incorrect paddle. [6]Total NNOB performed during the test. [7]Total amount of NNOB performed on all paddles combined. [8]NNOB performed around and above both feeders. [9]NNOB performed at the left feeder. [10]NNOB performed at the right feeder. [11]Head is still and not interacting with any object and can be coded with or without locomotion. [12]NNOB performed within the no choice space of the testing arena indicating no choice between feeders.

Table 3.2. Pig (n=11) performance behaviors during the paired learning phase of testing (Session 12-14). The formats consist of outside (CIIC), Inside (ICCI), and alternate (CICI, or ICIC).

	Format				Session				*P*-values		
	Outside	Inside	Alternate	SEM[1]	12	13	14	SEM[1]	Format	Session	Format x Session
Rate, no./min											
Eat[3]	1.01	1.2	1.01	±0.26	1.0	1.1	0.73	±0.18	0.15	0.26	0.27
NNOB Correct Pad[4]	4.1	5.0	3.8	±0.64	4.2	4.7	4.0	±0.43	0.21	0.52	0.89
NNOB Incorrect Pad[5]	3.4	1.8	2.0	±0.56	3.0[a]	2.4[ab]	1.7[b]	±0.35	0.08	0.01	0.36
Total NNOB[6]	31.4	28.7	24.4	±5.03	32.7	31.6[a]	20.2[b]	±3.16	0.37	0.004	0.43
NNOB Paddle[7]	7.5	6.7	5.8	±0.91	7.2	7.1	5.7	±0.62	0.30	0.14	0.75
NNOB Around[8]	7.1	5.7	4.5	±2.29	6.9	6.5	3.9	±1.31	0.61	0.08	0.71
NNOB Left[9]	7.2	6.0	6.0	±1.43	6.9	7.5	4.8	±0.96	0.76	0.10	0.30
NNOB Right[10]	5.4	5.7	4.1	±0.77	5.7	5.4	4.1	±0.73	0.33	0.23	0.34
Head Still[11]	5.4	3.0	4.7	±0.93	5.4	5.6	5.0	±0.50	0.39	0.42	0.46
No Choice NNOB[12]	0.9	1.8	1.1	±0.68	1.3	1.2	1.3	±0.35	0.51	1.00	0.69
Latency, %											
Eat[2,3]	11.0	9.1	11.4	±1.84	9.0	9.5	13.4	±2.49	0.57	0.50	0.64
NNOB Correct Pad[4]	3.0	2.2	3.5	±0.92	3.2	2.1	3.3	±0.70	0.37	0.41	0.55
NNOB Incorrect Pad[5]	18.5	18.6	12.7	±11.1	16.7	11.7	21.4	±7.82	0.83	0.64	0.78
Total NNOB[6]	2.9	1.8	2.2	±0.61	2.3	1.9	2.7	±0.34	0.38	0.07	0.72
NNOB Paddle[2,7]	2.5	1.7	2.4	±0.47	2.4	1.8	2.5	±0.35	0.38	0.15	0.84
NNOB Around[8]	21.5	40.1	38.7	±14.5	31.2	26.3	42.8	±10.63	0.56	0.51	0.66
NNOB Left[2,9]	2.5	5.5	2.9	±1.80	4.0	3.3	3.0	±1.19	0.51	0.63	0.63
NNOB Right[10]	14.8	4.4	10.4	±5.47	8.4	8.6	12.6	±4.45	0.32	0.76	0.24
Head Still[2,11]	5.9	4.0	4.7	±1.40	6.67	3.22	5.11	±1.19	0.58	0.07	0.43
No Choice NNOB[12]	50.1	40.7	56.8	±15.1	49.5	38.4	59.7	±8.72	0.56	0.10	0.90
Duration, %											
Eat[3]	17.1	22.6	14.5	±9.93	9.1[a]	15.8[ab]	29.4[b]	±5.96	0.72	0.02	0.44
NNOB Correct Pad[4]	24.3	40.6	34.0	±7.09	28.8	40.8	29.3	±4.55	0.24	0.07	0.89
NNOB Incorrect Pad[5]	12.5	6.8	8.9	±2.73	13.6	9.0[ab]	5.6[b]	±1.76	0.29	0.01	0.09
Total NNOB[6]	64.7	63.5	55.8	±11.3	64.7	70.2[a]	49.0[b]	±6.99	0.74	0.04	0.60
NNOB Paddle[7]	36.8	47.3	42.9	±7.04	42.4	49.7	34.9	±5.00	0.50	0.11	0.62
NNOB Around[8]	13.9	8.1	6.4	±3.65	11.1	10.23	7.03	±2.30	0.30	0.29	0.96
NNOB Left[9]	25.2	28.3	27.0	±4.14	25.1	29.5	25.9	±4.68	0.83	0.81	0.50
NNOB Right[10]	25.6	27.1	22.4	±6.62	28.5	30.4	16.1	±4.60	0.71	0.09	0.99
Head Still[11]	22.4	21.5	16.5	±4.0	21.6	20.2	18.6	±2.59	0.39	0.66	0.24
No Choice NNOB[12]	6.9	12.6	17.4	±6.90	13.5	9.9	13.4	±4.37	0.49	0.72	0.99

[1]Largest Standard Error Mean; [2]Non-parametric data that became normal upon log transformation. Data was then transformed back. [3]Head over bowl and consuming feed after reward is dispensed. [4]Non-nutritive oral behaviors associated with the correct paddle (i.e. walking or standing without any sniffing, drinking, chomping, licking, rooting, rubbing using the mouth, snout, or face). [5]NNOB associated with the incorrect paddle. [6]Total NNOB performed during the test. [7]Total amount of NNOB performed on all paddles combined. [8]NNOB performed around and above both feeders. [9]NNOB performed at the left feeder. [10]NNOB performed at the right feeder. [11]Head is still and not interacting with any object and can be coded with or without locomotion. [12]NNOB performed within the no choice space of the testing arena indicating no choice between feeders.

Table 3.3. Pig (n=10) performance behaviors performed by pigs classified as either dominant or submissive based on dominance indices in Table 3.8. Session 1 was a novel paired exposure followed by sessions 2 and 3 of paired conditioning. Sessions 4 and 5 were both individual conditioning exposures.

	Status			Session						*P*-Values		
	Dominant	Submissive	SEM[1]	1	2	3	4	5	SEM[1]	Status	Session	Status x Session
Rate, no./min												
Eat[2,3]	0.8	0.8	±0.16	0.9	0.4	1.0	0.7	0.9	±0.22	0.95	0.36	0.83
Total NNOB[6]	41.8	49.1	±5.07	40.1	52.4	49.4	50.6	34.8	±5.74	0.34	0.08	0.49
NNOB Paddle[7]	6.0	5.4	±0.54	3.1^{a}	6.6^{b}	6.0^{b}	5.9^{b}	6.9^{b}	±0.73	0.52	0.003	0.57
NNOB Around[8]	8.7	11.5	±1.35	11.4	12.1	11.7	10.3	5.2	±2.02	0.52	0.11	0.78
NNOB Left[9]	6.3	8.9	±0.91	8.2	9.0	8.6	7.6	4.7	±1.30	0.08	0.15	0.81
NNOB Right[10]	6.7	6.0	±0.65	5.4	6.9	7.9	5.85	5.77	±1.02	0.42	0.43	0.38
Head Still[11]	2.8	3.1	±0.41	2.7^{ac}	4.4^{b}	4.0^{bc}	2.05^{a}	1.6^{a}	±0.48	0.55	0.0001	0.44
No Choice NNOB[12]	0.4	0.7	±0.13	0.6^{a}	1.2^{a}	1.0^{a}	0.01^{b}	0.03^{b}	±0.16	0.12	<0.000	0.19
Latency, %												
Eat[3]	23.5	26.0	±2.83	52.2	15.1	26.8	15.0	14.5	±5.4	0.88	0.11	0.95
Total NNOB[2,6]	1.7	1.6	±0.53	2.0	1.4	2.9	1.2	1.4	±0.58	0.97	0.97	0.82
NNOB Paddle[2,7]	1.7	1.9	±0.62	2.4	1.4	3.6	1.2	1.5	±0.74	0.84	0.84	0.56
NNOB Around[8]	19.3	17.6	±4.52	27.3	13.6	16.2	8.7	26.5	±9.09	0.80	0.58	0.33
NNOB Left[2,9]	4.1	3.1	±0.62	10.5^{a}	3.5^{b}	5.0^{ab}	1.6^{b}	1.9^{b}	±1.41	0.28	0.001	0.84
NNOB Right[2,10]	2.6	3.6	±1.09	2.5	1.7	5.3	2.8	4.6	±1.51	0.53	0.31	0.81
Head Still[2,11]	2.9	2.7	±0.64	2.3	2.7	3.0	2.0	4.9	±0.87	0.20	0.83	0.73
No Choice NNOB[2,12]	0.4	0.7	±0.13	0.6^{a}	1.2^{a}	1.0^{a}	0.01^{b}	0.03^{b}	±0.16	0.12	<0.000	0.19
Duration, %												
Eat[3]	22.7	20.6	±2.85	25.54	11.5	29.3	20.6	21.5	±5.67	0.61	0.30	0.65
Total NNOB[6]	60.8	61.6	±2.74	51.7^{a}	69.1^{a}	49.9^{a}	66.8^{a}	68.6^{a}	±5.47	0.83	0.04	0.33
NNOB Paddle[7]	44.8	41.9	±3.52	23.2^{a}	47.0^{bc}	29.9^{ac}	54.7^{b}	61.9^{b}	±5.42	0.57	<0.000	0.05
NNOB Around[8]	10.1	12.6	±1.42	14.3	12.7	11.3	11.9	6.6	±2.38	0.24	0.25	0.44
NNOB Left[9]	27.4	31.0	±2.34	20.4^{a}	32.7^{b}	22.1^{ab}	35.5^{b}	35.4^{b}	±3.57	0.32	0.01	0.45
NNOB Right[10]	27.4	23.5	±2.02	17.1^{a}	26.9^{ab}	19.1^{a}	31.1^{b}	33.1^{b}	±3.45	0.21	0.01	0.11
Head Still[11]	15.1	15.8	±1.71	21.8^{a}	18.6^{a}	17.7^{ac}	10.8^{bc}	8.4^{b}	±2.04	0.77	<0.000	0.63
No Choice NNOB[12]	5.9	7.2	±1.51	14.2^{a}	9.5^{a}	8.7^{a}	0.2^{b}	0.1^{b}	±1.89	0.58	<0.000	0.02

[1]Largest Standard Error Mean; [2]Non-parametric data that became normal upon log transformation. Data was then transformed back. [3]Head over bowl and consuming feed after reward is dispensed. [4]Non-nutritive oral behaviors associated with the correct paddle (i.e. walking or standing without any sniffing, drinking, chomping, licking, rooting, rubbing using the mouth, snout, or face). [5]NNOB associated with the incorrect paddle. [6]Total NNOB performed during the test. [7]Total amount of NNOB performed on all paddles combined. [8]NNOB performed around and above both feeders. [9]NNOB performed at the left feeder. [10]NNOB performed at the right feeder. [11]Head is still and not interacting with any object and can be coded with or without locomotion. [12]NNOB performed within the no choice space of the testing arena indicating no choice between feeders.

Table 3.4. Pig (n=10) performance behaviors performed by pigs classified as either dominant or submissive based on dominance indices in Table 3.8. These sessions consist of the individual acquisition phase of testing (Sessions 6-11).

	Status			Session							*P*-Values		
	Dominant	Submissive	SEM[1]	6	7	8	9	10	11	SEM[1]	Status	Session	Status x Session
Rate, no./min													
Eat[3]	0.63	0.61	±0.04	0.8	0.6	0.7	0.5	0.6	0.6	±0.06	0.62	0.08	0.59
NNOB Correct Pad[4]	2.5	2.9	±0.27	3.1^{a}	2.8^{ab}	3.0^{a}	2.5^{ab}	1.8^{b}	3.1^{a}	±0.32	0.31	0.012	0.29
NNOB Incorrect Pad[5]	1.9	1.6	±0.21	2.5^{a}	2.2^{a}	1.8^{ab}	1.2^{b}	1.0^{b}	1.7^{ab}	±0.28	0.30	0.001	0.61
Total NNOB[6]	10.0	8.2	±0.97	12.5^{a}	11.2^{a}	8.5^{ab}	7.9^{b}	5.2^{b}	9.1^{ab}	±1.19	0.22	0.001	0.30
NNOB Paddle[7]	2.3	1.7	±0.24	3.2^{a}	1.8^{ab}	2.5^{ab}	1.3^{b}	1.6^{b}	1.7^{ab}	±0.39	0.08	0.01	0.65
NNOB Around[8]	2.0	1.6	±0.16	2.5	1.7	2.3	1.2	1.6	1.7	±0.31	0.09	0.07	0.64
NNOB Left[9]	4.6	3.8	±0.39	6.2^{a}	5.9^{ac}	3.3^{bc}	3.8^{ab}	2.3^{b}	3.6^{ab}	±0.66	0.18	0.001	0.06
NNOB Right[10]	4.1	3.5	±0.72	4.5	3.9	4.4	3.2	2.5	4.3	±0.77	0.55	0.18	0.84
Head Still[11]	2.1	2.0	±0.11	2.4^{ab}	1.9^{ab}	2.1^{ab}	1.7^{ab}	1.5^{a}	2.5^{b}	±0.22	0.55	0.02	0.88
No Choice NNOB[12]	0.13	0.01	±0.04	0.12	0.07	0.11	0.02	0.05	0.06	±0.05	0.04	0.57	0.42
Latency, %													
Eat[2,3]	10.6	11.3	±1.78	22.4^{a}	8.9^{b}	8.2^{b}	8.5^{b}	8.9^{b}	8.8^{b}	±3.18	0.80	0.02	0.72
NNOB Correct Pad[4]	3.0	2.4	±0.52	4.7^{a}	3.1^{ab}	3.1^{ab}	1.7^{b}	1.7^{b}	1.8^{b}	±0.63	0.44	0.003	0.77
NNOB Incorrect Pad[5]	10.5	14.6	±3.10	7.3	5.5	12.6	13.3	14.7	22.0	±6.30	0.37	0.54	0.29
Total NNOB[6]	2.0	1.6	±0.16	2.5	1.7	2.3	1.2	1.6	1.7	±0.31	0.09	0.07	0.64
NNOB Paddle[7]	2.3	1.7	±0.24	3.2^{a}	1.8^{ab}	2.5^{ab}	1.3^{b}	1.6^{b}	1.7^{ab}	±0.39	0.08	0.01	0.65
NNOB Around[8]	28.2	43.4	±10.61	18.9	31.3	38.1	30.6	53.2	42.4	±11.24	0.34	0.17	0.42
NNOB Left[2,9]	2.7	3.3	±0.49	3.1	3.4	3.0	1.9	2.8	4.1	±1.07	0.77	0.77	0.39
NNOB Right[10]	16.0	10.4	±2.96	11.0	7.9	19.6	14.5	16.0	10.2	±4.31	0.21	0.39	0.40
Head Still[11]	15.5	14.0	±2.90	11.7	8.2	17.6	23.7	15.5	11.6	±4.54	0.73	0.20	0.71
No Choice NNOB[12]	20.4	3.1	±6.85	6.4	16.2	8.7	7.7	13.7	17.8	±8.81	0.11	0.88	0.61
Duration, %													
Eat[3]	43.9	42.0	±3.81	30.1^{a}	41.1^{ac}	43.3^{abc}	47.9^{b}	59.7^{b}	35.8^{ac}	±4.64	0.74	0.0003	0.73
NNOB Correct Pad[4]	23.5	35.7	±2.52	31.4	28.7	33.1	30.2	21.8	32.6	±4.22	0.01	0.44	0.35
NNOB Incorrect Pad[5]	9.4	7.3	±1.03	10.4	9.6	7.1	6.9	5.3	10.6	±1.50	0.19	0.06	0.95
Total NNOB[6]	43.4	48.1	±2.72	54.4^{a}	49.2^{ab}	45.8^{ab}	42.4^{ab}	31.5^{b}	51.1^{a}	±4.38	0.26	0.01	0.60
NNOB Paddle[7]	32.9	43.0	±3.16	41.8	38.3	40.3	37.1	27.1	43.1	±4.64	0.05	0.162	0.37
NNOB Around[8]	9.5	5.1	±1.73	11.7^{a}	9.8^{a}	5.1^{a}	5.1^{a}	4.3^{a}	7.7^{a}	±2.06	0.11	0.03	0.46
NNOB Left[9]	22.8	23.8	±2.93	33.3^{a}	25.3^{ab}	18.0^{b}	22.2^{ab}	18.9^{b}	22.0^{ab}	±3.80	0.80	0.04	0.97
NNOB Right[10]	19.6	24.2	±1.91	20.2^{ab}	22.7^{ab}	27.4^{a}	19.9^{ab}	12.5^{b}	28.8^{a}	±2.93	0.13	0.003	0.19
Head Still[11]	11.3	7.5	±1.83	13.1^{a}	8.1^{ab}	9.0^{ab}	7.8^{ab}	7.3^{b}	11.3^{ab}	±1.82	0.19	0.03	0.76
No Choice NNOB[12]	1.0	0.02	±0.38	0.84	1.12	0.44	0.26	0.18	0.30	±0.51	0.10	0.69	0.79

[1]Largest Standard Error Mean; [2]Non-parametric data that became normal upon log transformation. Data was then transformed back. [3]Head over bowl and consuming feed after reward is dispensed. [4]Non-nutritive oral behaviors associated with the correct paddle (i.e. walking or standing without any sniffing, drinking, chomping, licking, rooting, rubbing using the mouth, snout, or face). [5]NNOB associated with the incorrect paddle. [6]Total NNOB performed during the test. [7]Total amount of NNOB performed on all paddles combined. [8]NNOB performed around and above both feeders. [9]NNOB performed at the left feeder. [10]NNOB performed at the right feeder. [11]Head is still and not interacting with any object and can be coded with or without locomotion. [12]NNOB performed within the no choice space of the testing arena indicating no choice between feeders.

Table 3.5. Pig (n=10) performance behaviors performed by pigs classified as either dominant or submissive based on dominance indices in Table 3.8. These sessions consist of the paired learning phase of testing (Sessions 12-14).

	Status			Session				*P*-Values		
	Dominant	Submissive	SEM[1]	12	13	14	SEM[1]	Status	Session	Status x Session
Rate, no./min										
Eat[3]	1.2	0.70	±0.16	1.1	0.98	0.79	±0.19	0.06	0.57	0.79
NNOB Correct Pad[4]	4.3	4.5	±0.44	4.3^{a}	4.8^{a}	4.2^{a}	±0.38	0.72	0.35	0.01
NNOB Incorrect Pad[5]	1.9	2.4	±0.43	2.7^{a}	2.2^{ab}	1.6^{b}	±0.37	0.40	0.03	0.26
Total NNOB[6]	13.5	13.5	±1.60	31.8^{a}	30.3^{a}	20.4^{b}	±1.30	0.10	0.004	0.004
NNOB Paddle[7]	6.2	7.0	±0.60	7.0^{a}	7.0^{a}	5.8^{b}	±0.56	0.39	0.16	0.02
NNOB Around[8]	6.5	4.6	±1.36	6.6^{a}	6.2^{a}	3.7^{b}	±1.14	0.35	0.03	0.07
NNOB Left[9]	6.79	5.66	±0.83	6.7^{a}	7.1^{a}	4.9^{b}	±0.83	0.37	0.10	0.04
NNOB Right[10]	4.78	5.23	±0.74	5.7^{a}	5.2^{a}	4.1^{b}	±0.69	0.68	0.16	0.04
Head Still[11]	4.8	6.2	±0.46	5.5	5.8	5.2	±0.41	0.07	0.39	0.36
No Choice NNOB[12]	0.9	1.9	±0.36	1.5	1.3	1.4	±0.30	0.07	0.91	0.34
Latency, %										
Eat[3]	9.0	11.8	±1.27	9.4	8.7	13.4	±2.24	0.16	0.42	0.49
NNOB Correct Pad[2,4]	3.3	2.0	±0.33	2.8	1.9	2.9	±0.38	0.02	0.11	0.53
NNOB Incorrect Pad[2,5]	5.8	7.3	±3.26	6.0	5.9	7.7	±3.03	0.74	0.85	0.60
Total NNOB[6]	2.7	1.7	±0.33	2.3	1.8	2.6	±2.71	0.07	0.08	0.80
NNOB Paddle[2,7]	2.7	1.7	±0.30	2.3	1.7	2.4	±0.29	0.05	0.12	0.79
NNOB Around[8]	33.3	34.9	±9.35	35.6	25.8	41.0	±9.23	0.91	0.41	0.08
NNOB Left[2,9]	3.2	4.1	±1.51	4.4	3.6	3.0	±1.21	0.68	0.42	0.49
NNOB Right[2,10]	5.6	4.2	±1.48	5.4	4.4	4.8	±1.6	0.52	0.89	0.64
Head Still[2,11]	5.9	3.9	±1.01	6.9	3.3	5.0	±1.11	0.20	0.06	0.93
No Choice NNOB[12]	58.6	35.4	±7.20	45.9	37.8	57.3	±6.90	0.05	0.08	0.27
Duration, %										
Eat[3]	20.2	16.4	±6.08	9.4^{a}	17.8^{ab}	27.6^{b}	±5.45	0.67	0.02	0.19
NNOB Correct Pad[4]	39.4	30.0	±4.57	30.2^{a}	42.1^{b}	31.8^{ab}	±4.17	0.18	0.04	0.28
NNOB Incorrect Pad[5]	7.9	9.6	±1.87	12.1^{a}	8.5^{ab}	5.8^{b}	±1.85	0.54	0.04	0.30
Total NNOB[6]	65.5	65.3	±3.18	67.7	70.5	58.01	±3.19	0.97	0.01	0.18
NNOB Paddle[7]	47.3	39.6	±4.16	42.3	50.5	37.6	±4.39	0.22	0.10	0.18
NNOB Around[8]	9.7	7.5	±2.51	10.3^{a}	9.2^{a}	6.2^{a}	±2.09	0.56	0.11	0.03
NNOB Left[9]	28.1	26.2	±2.47	24.4	29.1	27.9	±3.98	0.62	0.74	0.07
NNOB Right[10]	28.2	20.8	±2.84	28.2^{a}	30.6^{a}	15.9^{b}	±3.13	0.08	0.01	0.005
Head Still[11]	19.4	19.9	±2.67	20.7	19.1	19.2	±2.69	0.89	0.87	0.84
No Choice NNOB[12]	8.5	18.2	±3.81	15.1	10.8	14.2	±3.48	0.11	0.52	0.07

[1]Largest Standard Error Mean; [2]Non-parametric data that became normal upon log transformation. Data was then transformed back. [3]Head over bowl and consuming feed after reward is dispensed. [4]Non-nutritive oral behaviors associated with the correct paddle (i.e. walking or standing without any sniffing, drinking, chomping, licking, rooting, rubbing using the mouth, snout, or face). [5]NNOB associated with the incorrect paddle. [6]Total NNOB performed during the test. [7]Total amount of NNOB performed on all paddles combined. [8]NNOB performed around and above both feeders. [9]NNOB performed at the left feeder. [10]NNOB performed at the right feeder. [11]Head is still and not interacting with any object and can be coded with or without locomotion. [12]NNOB performed within the no choice space of the testing arena indicating no choice between feeders. [13]Point event of feed reward being released from feeder.

Table 3.6. Descriptive statistics and dominance indices for each pig (n=10)- results for the dominance indices calculations for each pair of pigs. Pen 13-11 was eliminated from this calculation due to a lack of sampling data.

	Pig ID	Pen	Index[1]	Frequency of Agonistic Interactions[2]	Mean	SD[3]	CV[4]	Min[5]	Max[6]
Dominant									
Indices	-	-	-	-	71.8	16.85	23.3	52.7	91.4
Freq of Agonistic Interactions	-	-	-	-	43.4	15.32	35.3	18.0	56.0
Memphis	3271	03-05	79.0	55	-	-	-	-	-
Jefe	3349	04-06	91.4	44	-	-	-	-	-
Padme	3348	08-10	55.6	18	-	-	-	-	-
Abbott	3281	09-07	52.7	44	-	-	-	-	-
Adrian	3276	14-12	80.3	56	-	-	-	-	-
Submissive									
Indices	-	-	-	-	28.2	16.85	59.7	8.6	47.3
Freq of Agonistic Interactions	-	-	-	-	43.4	15.32	35.3	18.0	56.0
Oscar	3302	03-05	21.0	55	-	-	-	-	-
Dulcina	3352	04-06	8.6	44	-	-	-	-	-
Leia	3344	08-10	44.4	18	-	-	-	-	-
Mateo	3275	09-07	47.3	44	-	-	-	-	-
Grayson	3264	14-12	19.7	56	-	-	-	-	-

[1]Dominance Index calculated for each pig; [2]Total number of times an agonistic interaction occurred; [3]Standard deviation; [4]Coefficient of variation; [5]Minimum measure; [6]Maximum measure.

Table 3.7. Categorical assessment of social status in relation to test motivation as expressed through paddle presses. Chi-square analysis $X^2 = (1, n=25) = 4.89$, $P = 0.03$.

	[1]Dominant	[2]Submissive
Pressed No Paddles [3]	9 (6.24)[5] [1.22][6]	4 (6.76) [1.13]
Pressed both paddles[4]	3 (5.76) [1.32]	9 (6.24) [1.22]

[1]Higher dominance index number than pen-mate as shown in table 3.8. [2]Lower dominance index number than pen-mate as shown in table 3.8. [3]Pig pushed zero of the paddles that resulted in the reward (freeloader). [4]Pig pushed 2 paddles to trigger all food rewards. [5]Expected values of chi-square analysis assuming equal distribution. [6]Residual error.

Figures

Figure 3.1. The CogShape feeder prototype consists of two separate hoppers and four total paddles. The left and right side are separated by a barrier. Each paddle can be programmed to dispense a feed reward into the bowl below upon one or multiple successful paddle pushes.

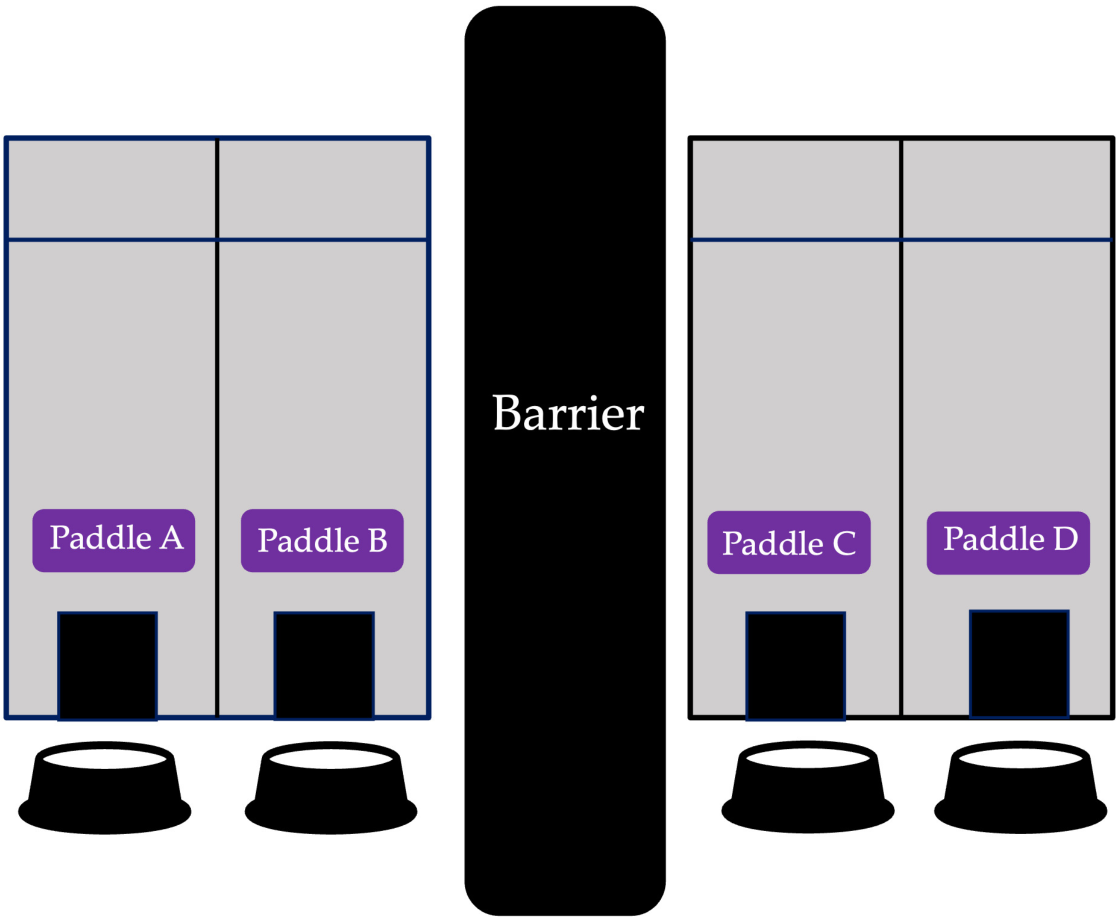


Figure 3.2. Diagram of formats used during feeder prototype testing. [1]The conditioning format was used from the novel pairs phase (session 1), to halfway through the individual acquisition phase (session 6-8). Starting in the latter half of the individual acquisition phase (9-11), each pen was randomly assigned one of the four patterns on the right. The black circles indicate activation. [2]A-Alternating had paddles A and C active. [3]Outside had the outer A and D paddles activated. [4]Inside had the inner two B and C paddles activated. [5]B-Alernating had the B and D paddles activated. Each pen kept their pattern for the remainder of the study.

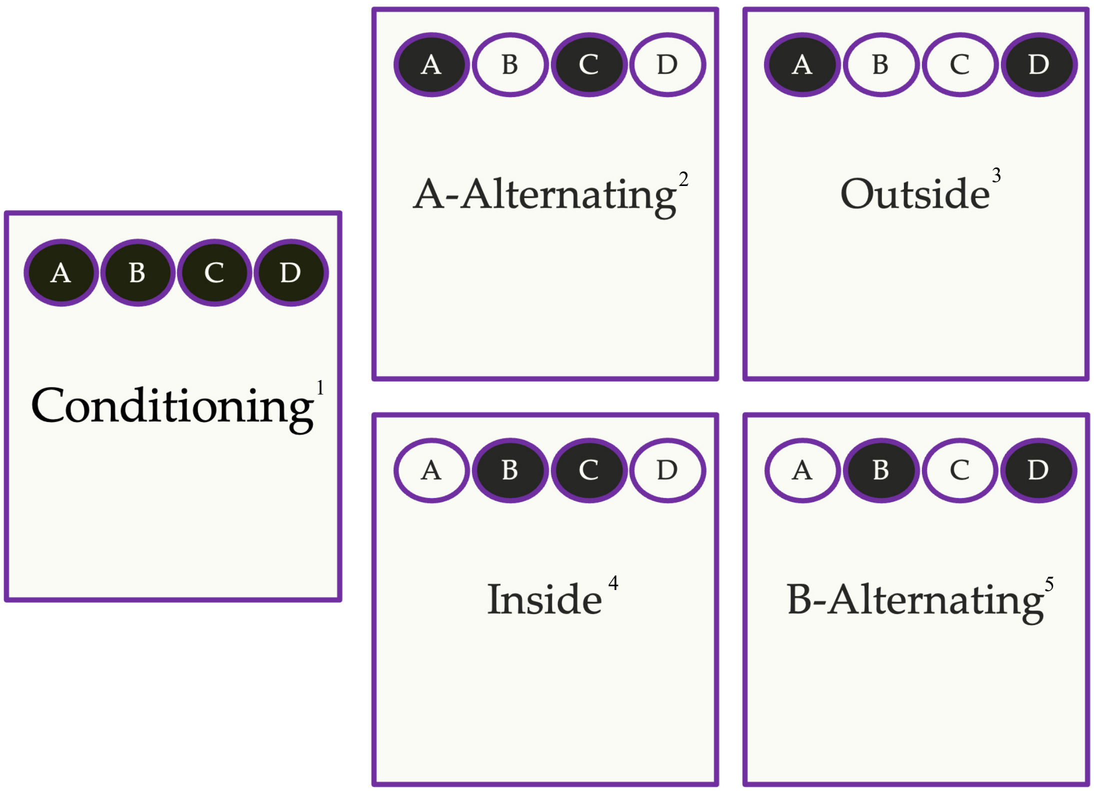


Figure 3.3. Decision making cycle for adjustments made to the feeder system and testing protocol. (Adapted from Martin & Bateson, 2007 p.30). Paired conditioning sessions began with an initial question of how it was best to utilize the feeder prototype. These measures where observed and data was collected. Based on the information collected during the paired conditioning sessions, hypotheses were formed, and protocol and methods were adjusted for subsequent sessions.

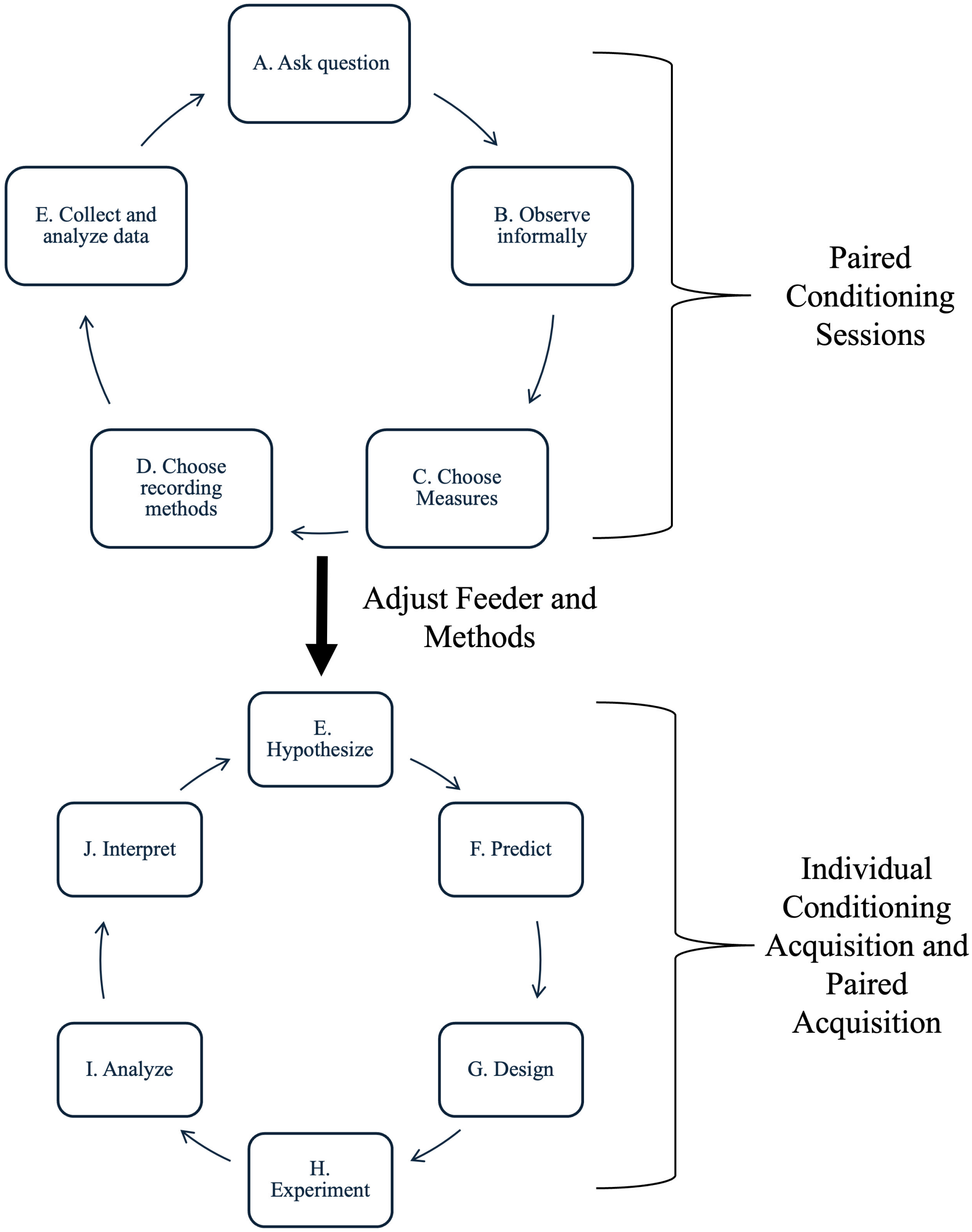


Figure 3.4. Recommended strategies for different testing various research goals during the feeder prototype test. [1]Contrafreeloading can be tested with increasing the number of paddle presses. [2]Cognition can be measured through reversal of assigned paddle format. [3]Social status can be assessed through the quantification of advance and retreat behaviors that can then be used to calculate dominance.

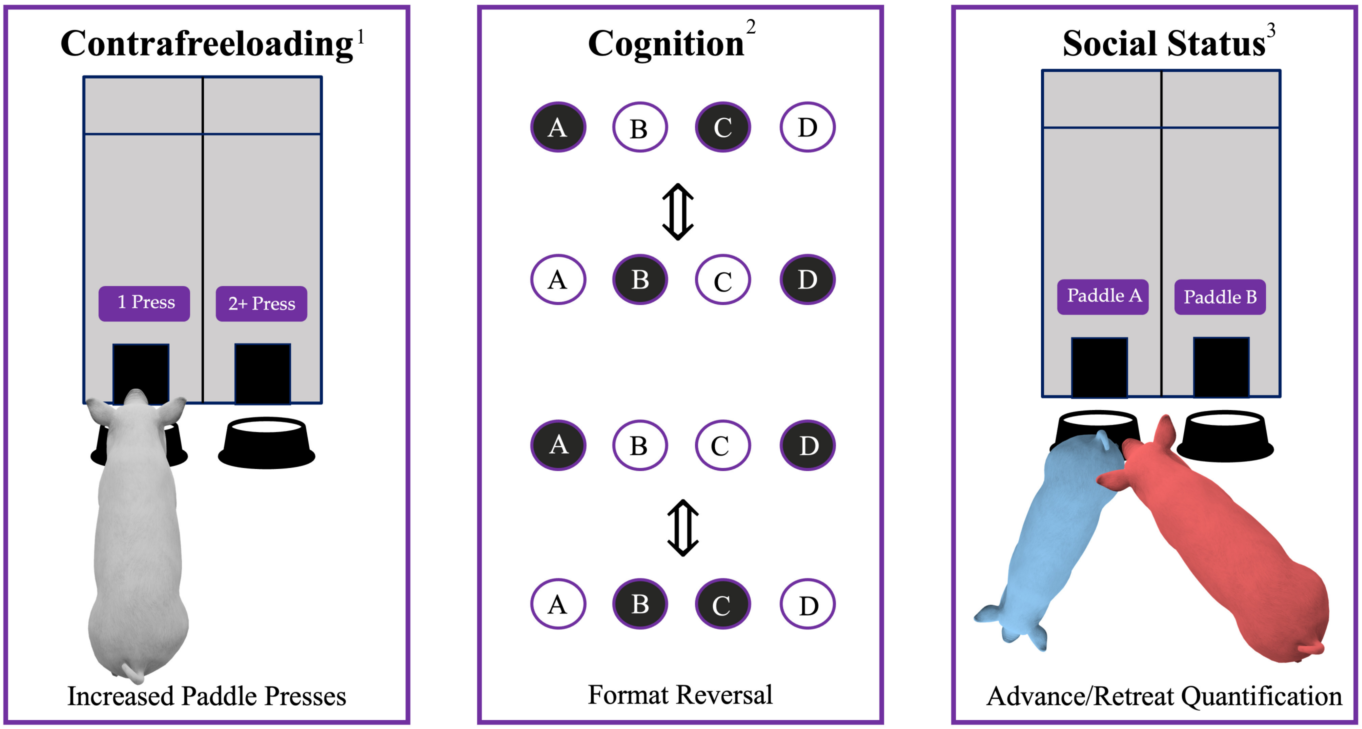


Figure 3.5. The spatial ethograms utilized on the feeder prototype study. The zones were coded whenever NNOB occurred in the corresponding area. The structural behavior of eat could only occur in the bowl zone. [1]Behavioral coding began once the pig crossed the dashed entrance line. The blue No Choice zone denotes the neutral area that does not indicate a choice between either feeder. [2]Spatial Ethogram B shows the spatial zones that were used for quantitative behavioral coding. As the colors denote a heat map the green zone is around the feeder and the least desired zone. The red zone is the most desired zone as it is the desired behavior to push the paddle.

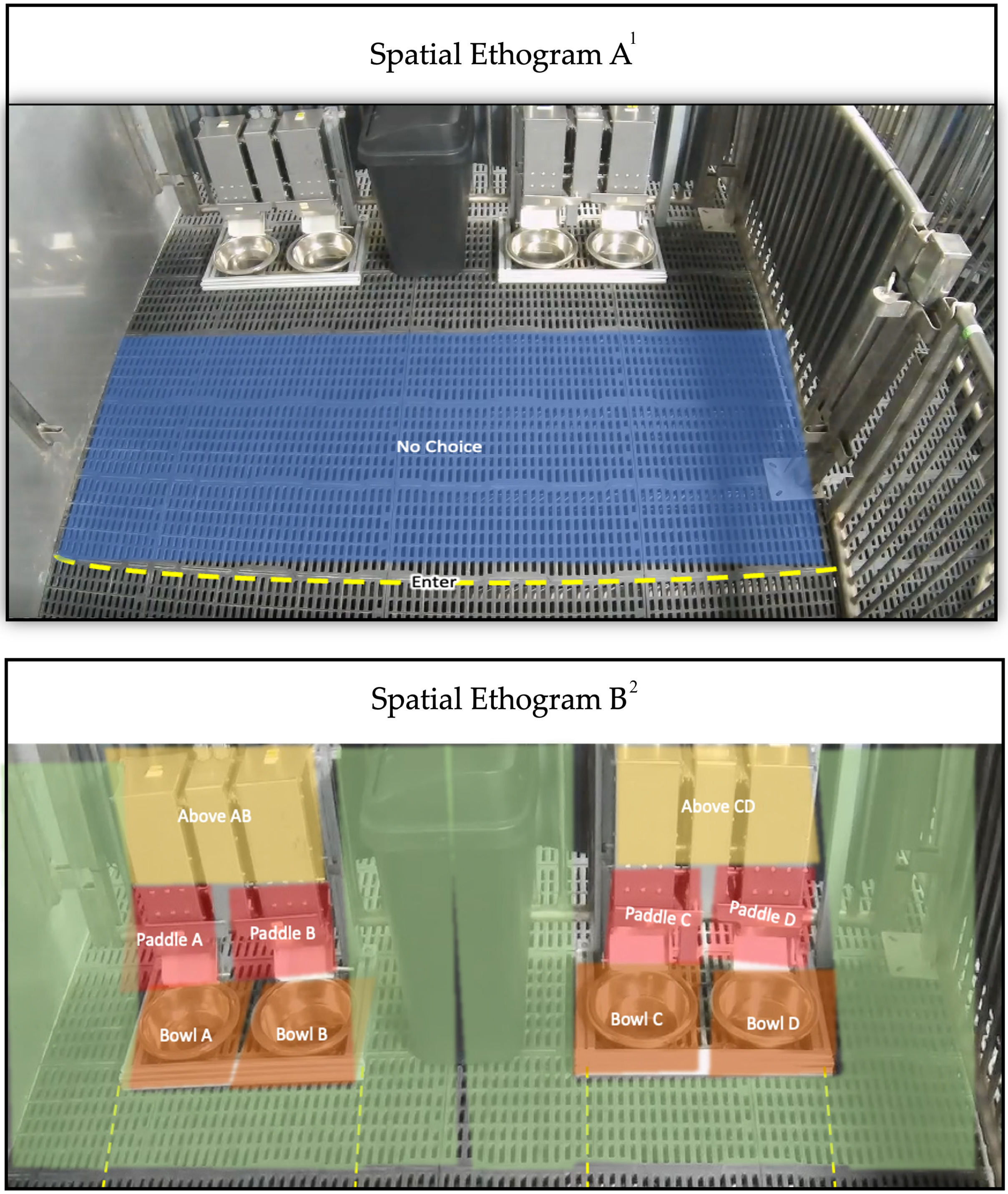


Figure 3.6. (A) Dominance flow chart outlines the formulation of social hierarchy (Adapted from DISC, Marston for animals). A pig that is attempting to become dominant may adapt a compliance-based strategy using aggressive behaviors to force their counterpart to into compliance and accept a subordinate position. The alternative is for the dominant pig to entice their counterpart into accepting the subordinate role using cooperative behaviors. (B) Aggressive and submissive behaviors recorded throughout the social relationship are recorded and plugged into the dominance index equation (Adapted from Martin & Bateson, 2007, p. 133; Zumpe & Michael, 1986). A number is acquired for both pigs and allows for assignment of dominant and submissive status. The percentage also allows for a scaling of the relationship.

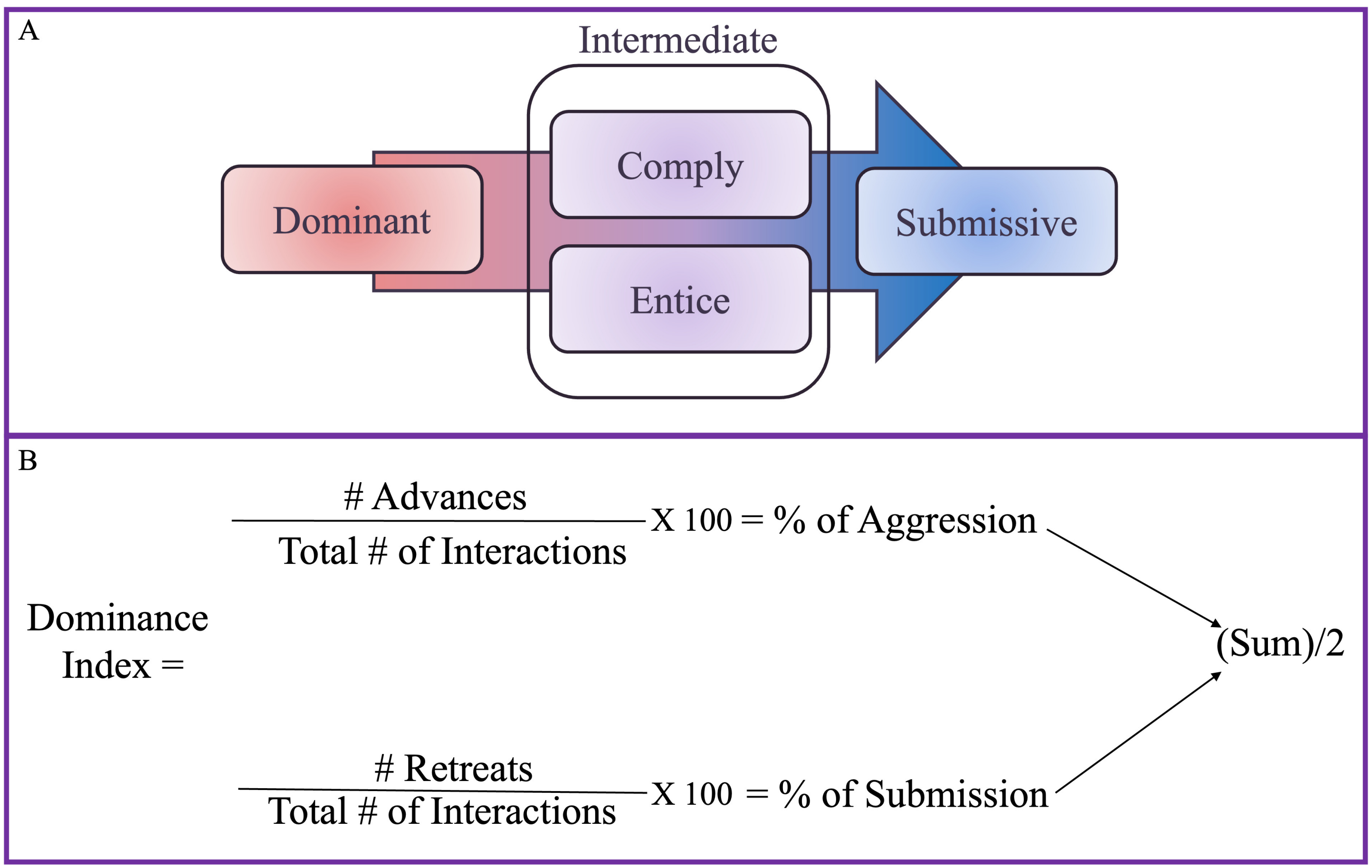


Figure 3.7. Results of timeline of feeder prototype study. For the novel session and all conditioning sessions (Sessions 1-5), every paddle triggered a food reward of 50 grams into each bowl. Each pen was randomly assigned a pattern starting at individual acquisition. The first three sessions of individual acquisition (Sessions 6-8) only two paddles were active, but feed was dispensed into all four bowls. For the latter half of individual acquisition (Sessions 9-11) only two paddles were active again and feed was only dispensed into two bowls. All formats were retained through the trained pair phase (Sessions 12-14).

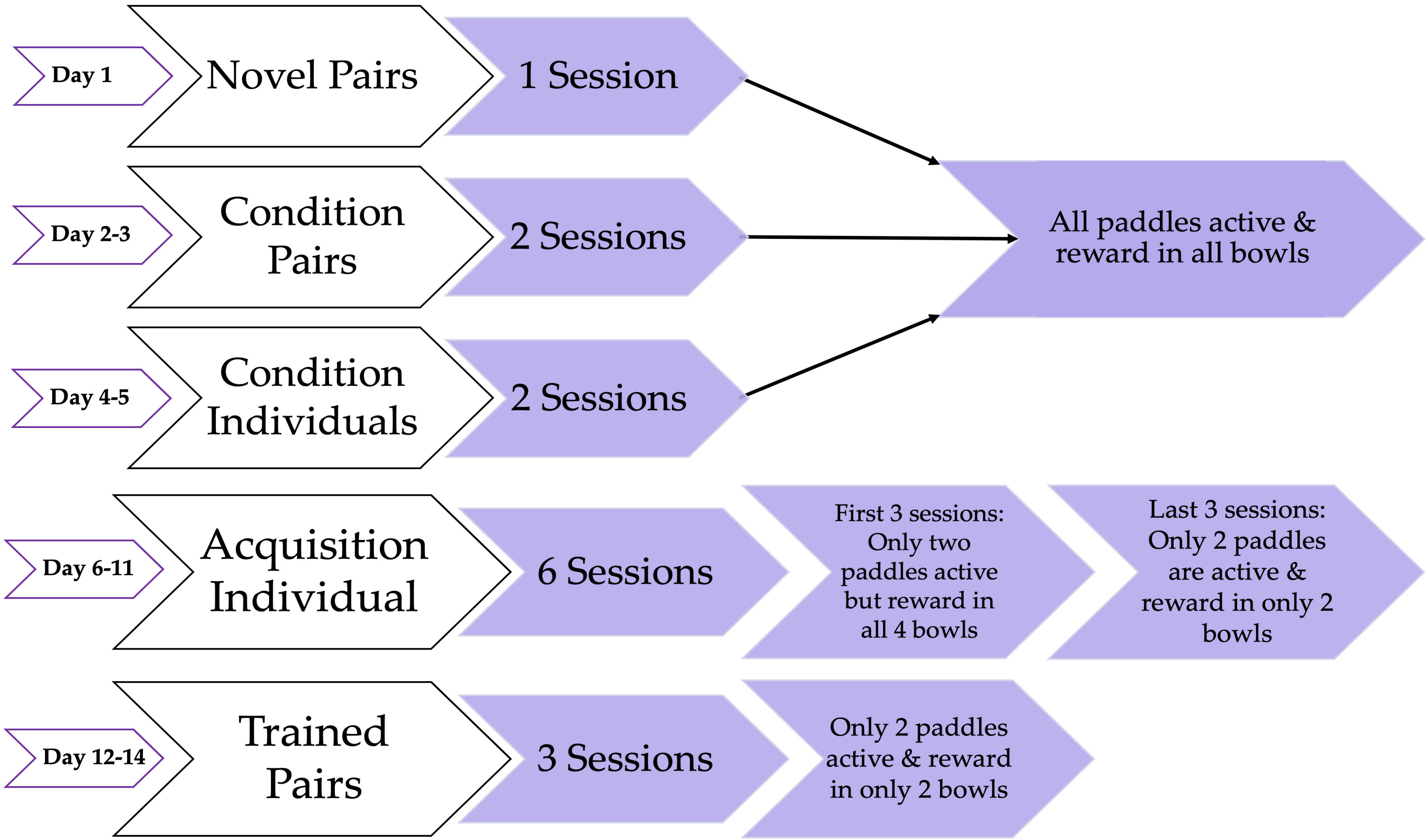


Figure 3.8. Line graph denoting pig (n=11) correct paddle contact by format during the individual learning phase of testing (session 6-11). The formats consist of outside (CIIC), Inside (ICCI), and alternate (CICI, or ICIC). Error bars represent SEM. (*$P \leq 0.002$; Tukey Kramer Adjustment) Error bars represent SEM.

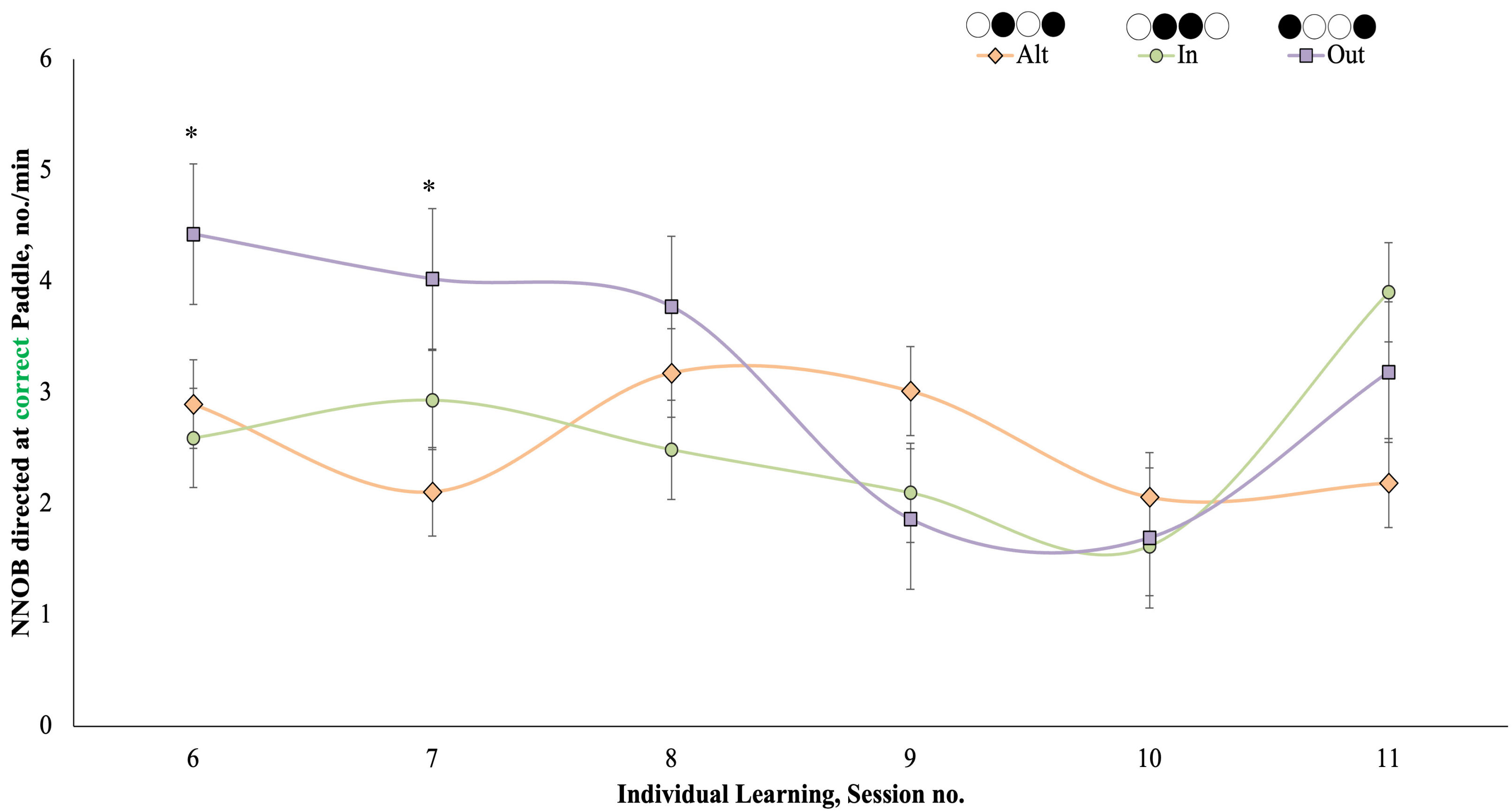


Figure 3.9. Bar graphs showing pig (n=11) non-nutritive oral behavior in contact with any paddle in relation to format (Alternate, Inside, Outside) during the individual acquisition phase of testing (Sessions 6-11). Error bars represent SEM. LS Means differ (**P* =0.005; ***P* =0.04; ****P* =0.03; Tukey-Kramer adjustment). Format*Session interaction (*P* =0.02).

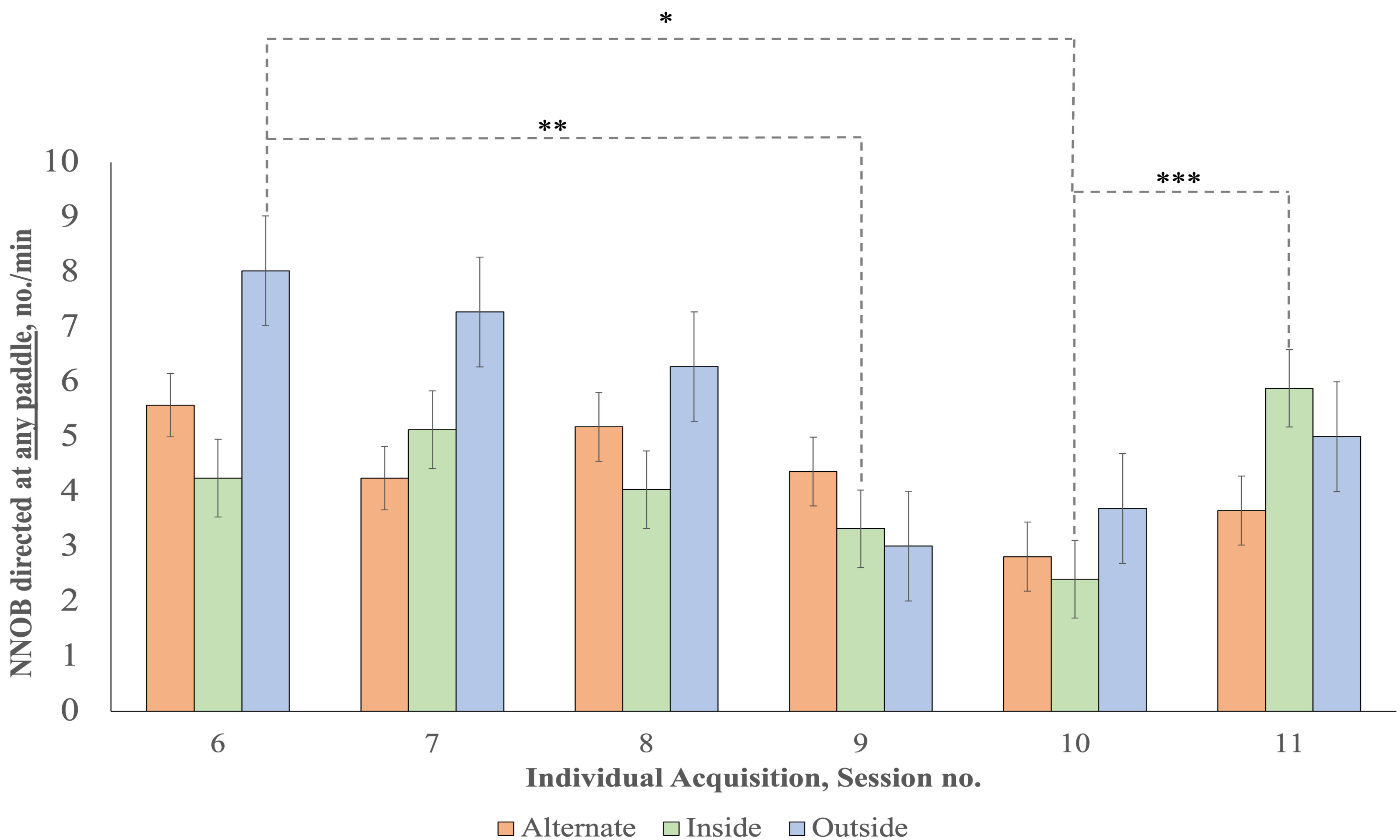


Figure 3.10. Bar graph representation of the calculated dominance indices from Table 3.8. Black line denotes total frequency of agonistic interactions by pen. Pigs (n=10), are arranged by pen on the x-axis.

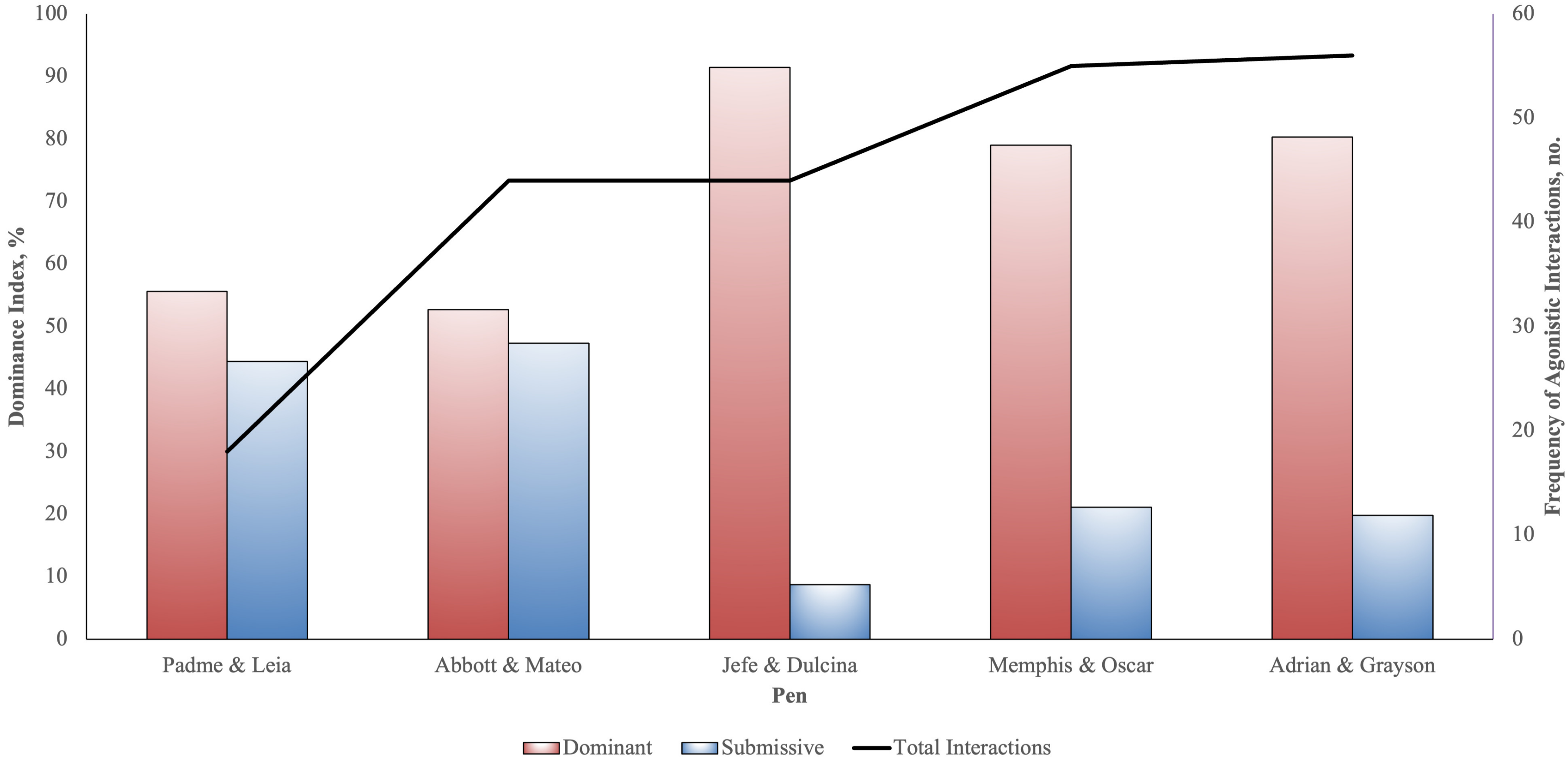
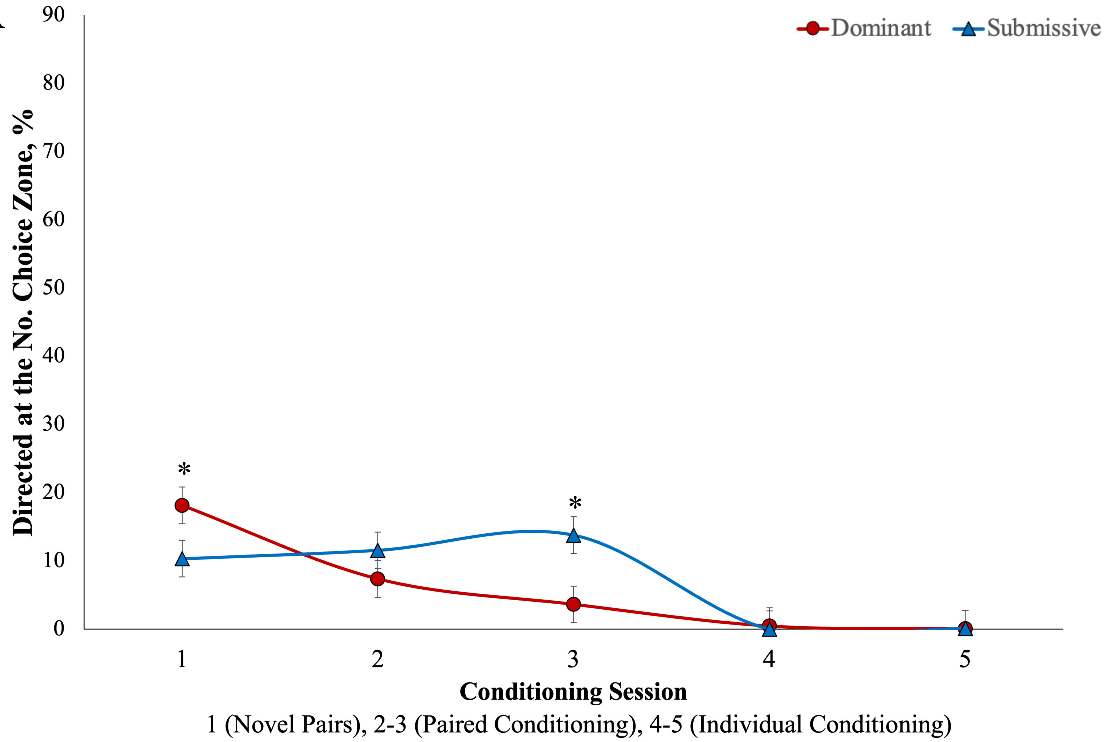


Figure 3.11. (A) Bar graph showing the duration of non-nutritive oral behavior performed in the no-choice zone of the testing arena in the conditioning phase of the study. *LS means differ ($P \leq 0.03$; Tukey-Kramer adjustment) Error bars represent SEM. (B) Bar graph showing duration of pig (n=11) non-nutritive oral contact with any paddle in the conditioning phase of the study. *LS means differ ($P \leq 0.002$; Tukey-Kramer adjustment). Status*Session interaction ($P = 0.05$).

A



B

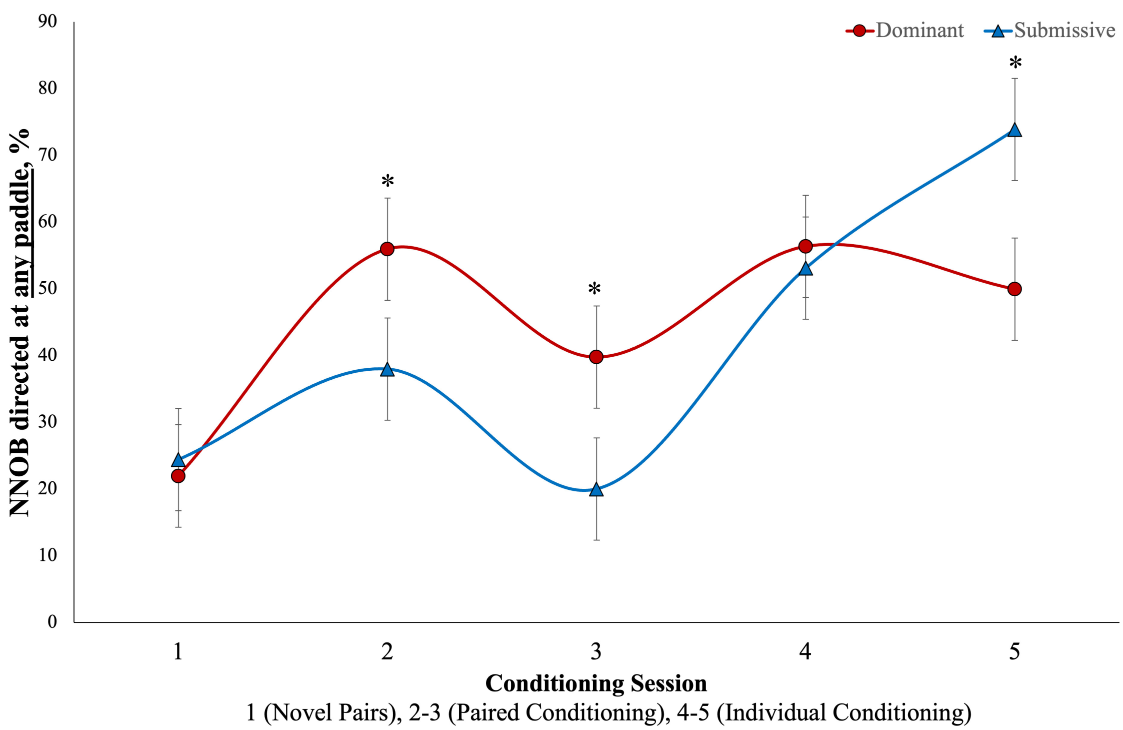


Figure 3.12. Bar graph of dominant and submissive pigs (n=10) contact with the correct paddle by rate (no./min). This is NNOB contact with the correct paddle according to pen format within the paired learning phase (Session 12-14). **P* =0.04 LS Means for rate between submissive pigs in session 12 and 13. Status*Session interaction of *P* =0.01. Error bars represent SEM.

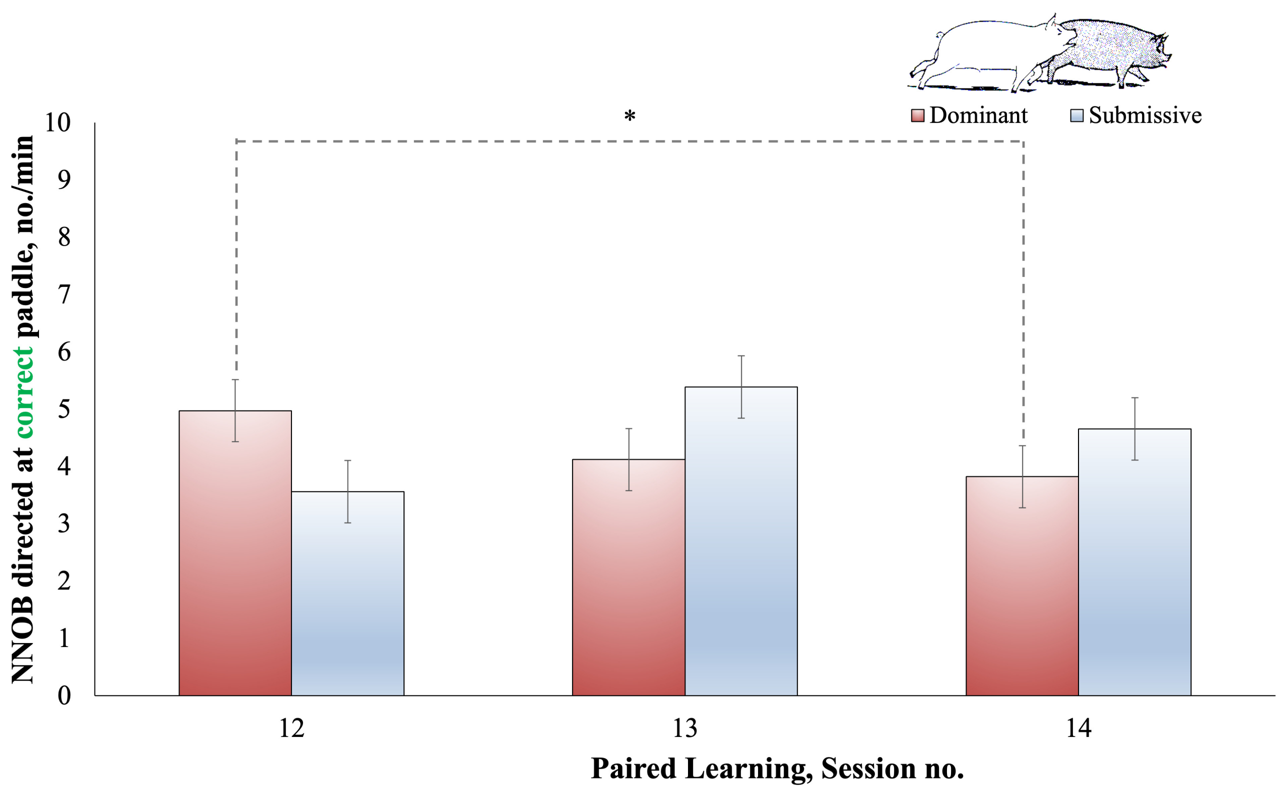


Figure 3.13. Bar graph of dominant and submissive pig (n=10) contact with any paddle by rate (no./min). This is NNOB contact with the correct paddle according to pen format within the paired learning phase (Session 12-14). **P* =0.05 LS Means for rate between dominant pigs in session 12 and 14. Status*Session interaction of *P* =0.02. Error bars represent SEM.

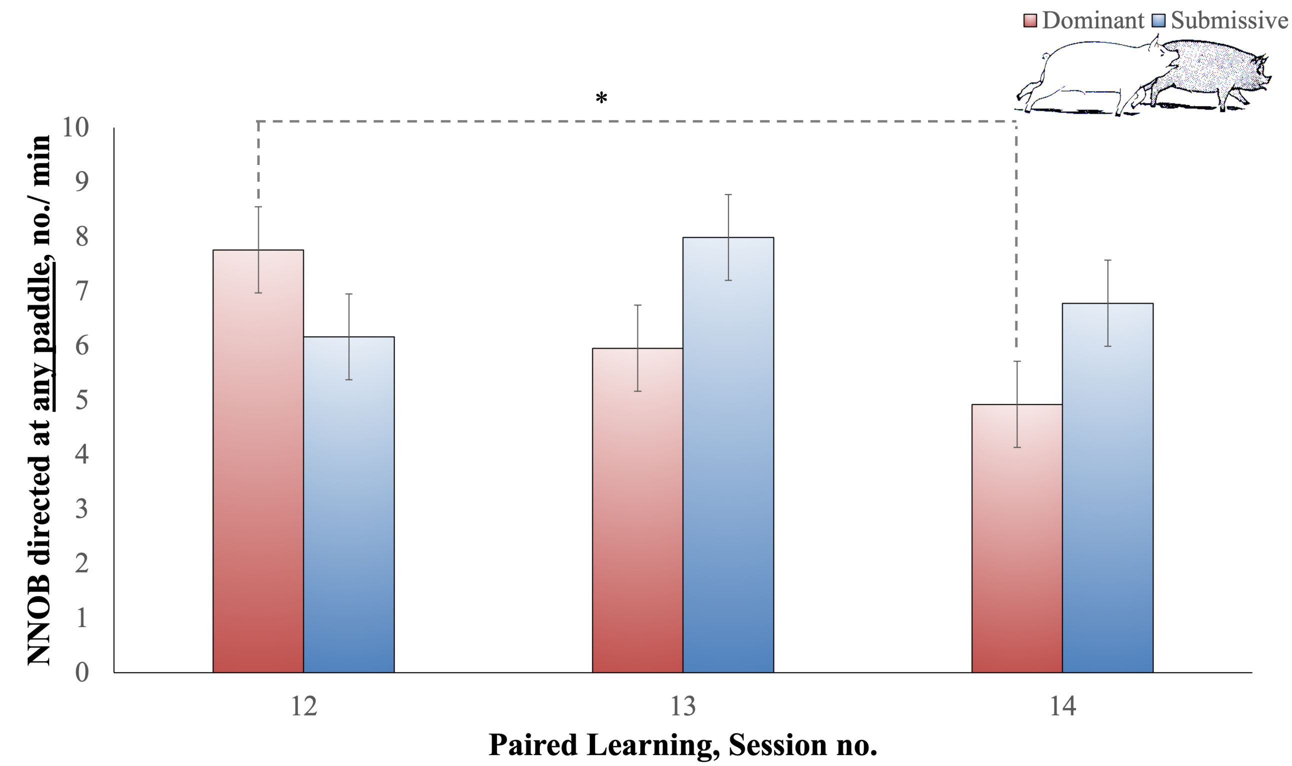


Figure 3.14. Bar graph illustrating rate of pig (n=11) non-nutritive oral behavior contact with the right or left feeder by dominant and submissive pigs in the paired learning phase of testing (Sessions 12-14). (B) Bar graphs showing duration of non-nutritive oral behavior in the zone around either feeder in the paired learning phase of testing (Sessions 12-14). (C) Bar graph showing total combined rate of non-nutritive oral behavior in the paired learning phase of testing (Sessions 12-14). *LS means differ (*P* =0.04). Status*Session interaction (*P* =0.04). The red graph represents the laterality of dominant pigs while the blue graph represents laterality of submissive pigs. Error bars represent SEM.

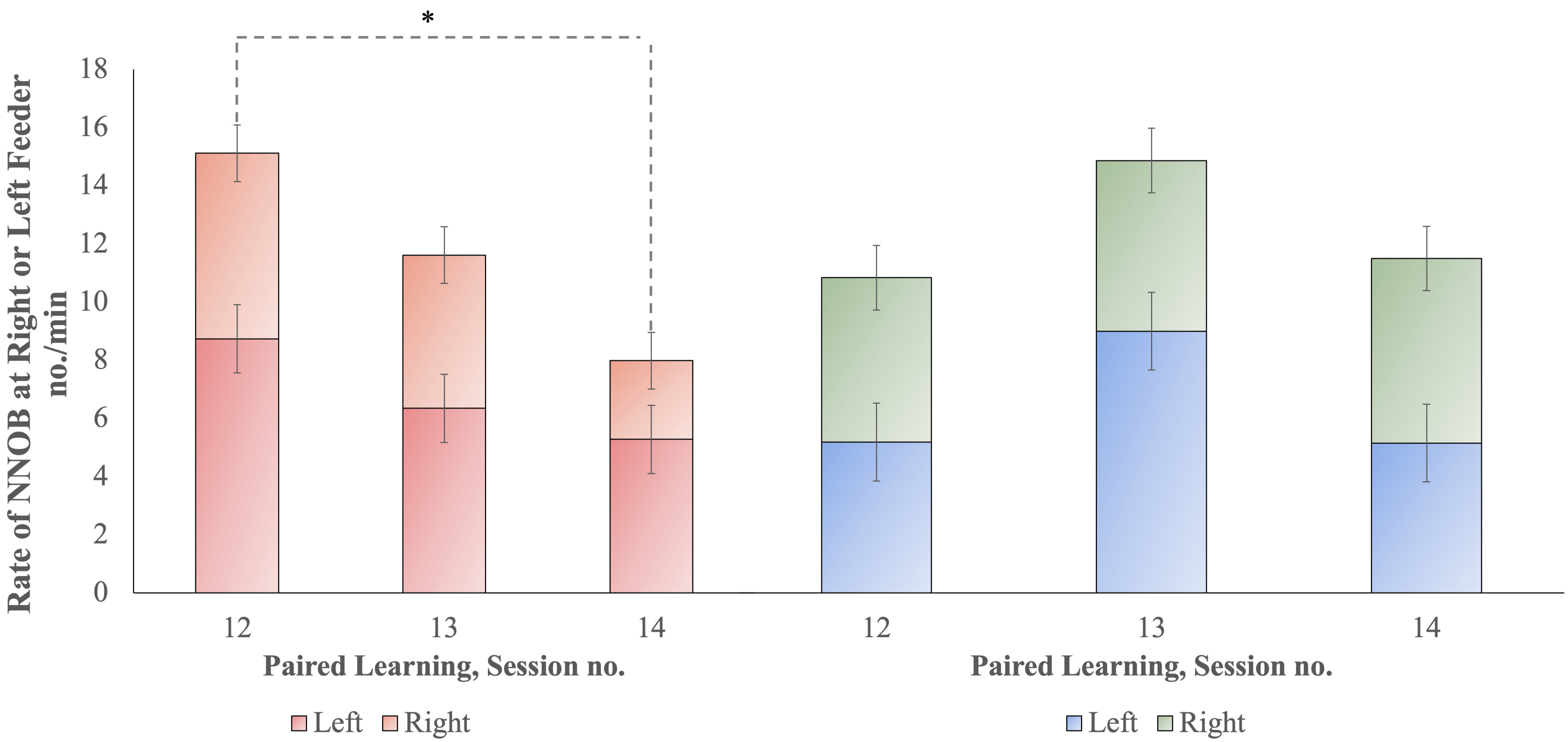
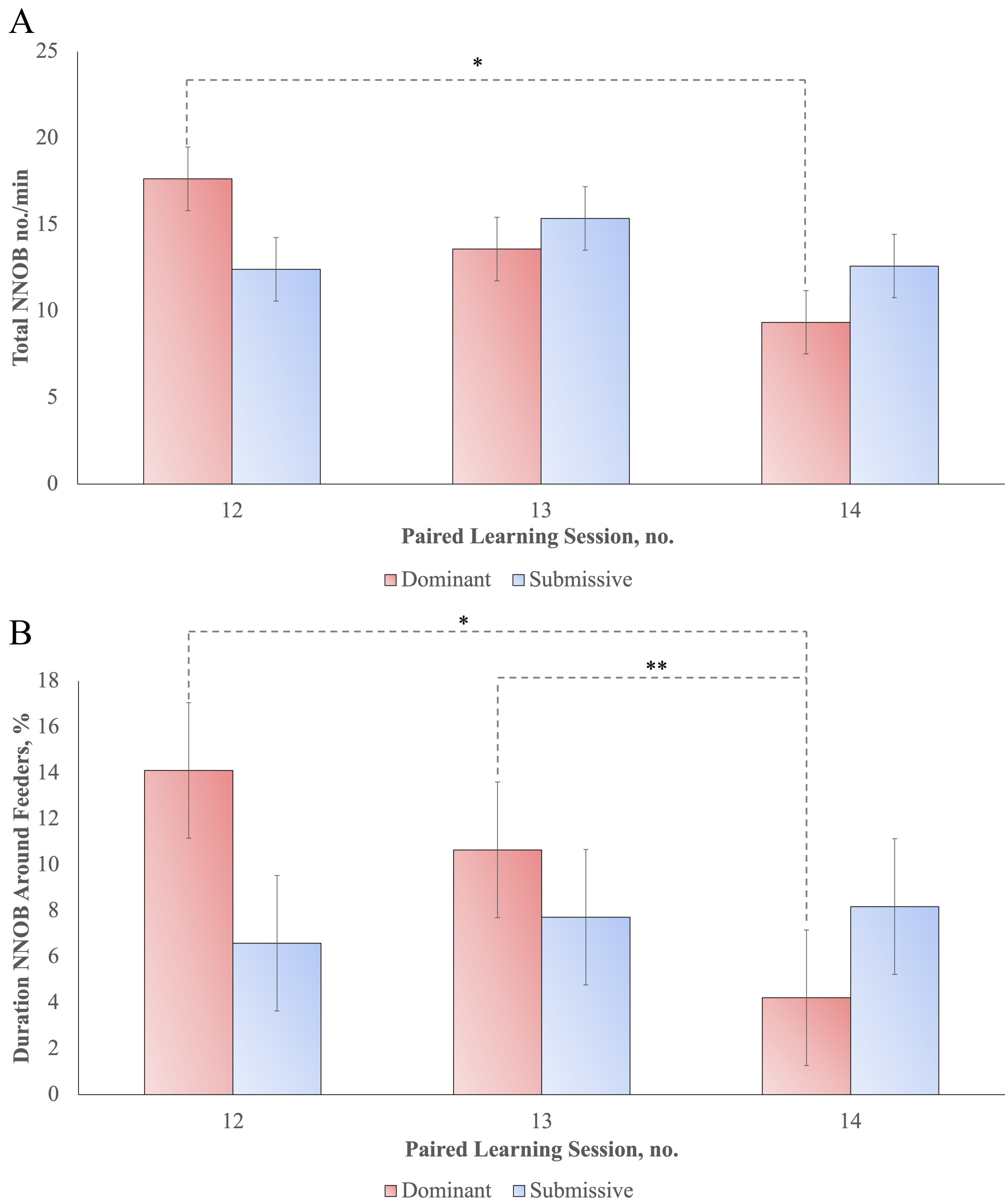


Figure 3.15. (A) Bar graph showing pig (n=11) rate of combined execution of non-nutritive oral behaviors in the paired learning phase of testing (Sessions 12-14). *LS Means differ P=0.001 with a social by session interaction (P =0.004). (B) Bar graph showing the duration of non-nutritive oral behaviors performed in the zones around the feeder. LS means differ (*P =0.002; **P =0.03; Tukey-Kramer adjustment). Status*Session interaction (P =0.03). Error bars represent SEM.



Chapter 4 - Influence of Social Status on Hematology Before and After Exposure to Cognitive Testing and a Fermented Sorghum Tea Preference Test

Abstract

The Ossabaw minipig has become the ideal model for translational research due to the many physiological similarities that are shared with humans. Due to the unique attributes of the Ossabaw there is a need for further publication of normal hematology results in order to establish a reliable baseline. Dominant and submissive pigs are known to deviate in size, stress and immune function so the relationship between social status (dominant and submissive) and metabolic blood parameters was explored. A secondary goal is to assess the potential health benefits of fermented sorghum tea consumption. The pigs (n=11; age 15.6 ±0.18 SEM months; barrows and gilts; pair-housed) were exposed to a 3-choice preference maze to evaluate preference for fermented sorghum teas (FST) as well as a cognitive feeder test to evaluate individual and paired learning. Blood was collected from the jugular vein the day before testing started and again on one of the last days of the trial. The samples were sent out to an external laboratory for testing of complete blood counts (CBC) and chemistry. These findings reveal the potential for physiological differentiation between dominant and submissive pigs that may be reflected in their behavior. Dominant pigs had a higher body weight overall when compared to the submissive pigs (P =0.03; 57.3 and 49.8 ±4.13, respectively). Dominant pigs experienced a significant drop in cholesterol from pre-study to post study (P =0.04; 85.3 and 77.0 ±3.69 SEM, respectively). Dominant pigs also had a tendency for higher plasma glucose and triglyceride levels (P =0.07; 61.7 and 51.3 ±4.59 SEM, respectively; P =0.09; 29.3 and 22.2 ±2.69 SEM,

respectively). This swine consisted of healthy pigs and while the conclusions based off this dataset are minimal the effects are likely to be amplified in a metabolically challenged herd.

These results show the potential for physiological differentiation based on social status that can have on subsequent hematology results. There is the potential for cholesterol reducing benefits stemming from the consumption of fermented sorghum tea. This being more greatly reflected in the dominant pigs as they had a tendency for higher tea consumption in milliliters (P =0.06; 918.0 and 872.4 ±16.18, respectively). Further work allowing for the collection of more longitudinal data on a larger number of lean and obese pigs will be necessary to validate these results.

Keywords: Ossabaw; Swine; Porcine; Hematology; Sorghum; Dominance; Behavior

Introduction

The Ossabaw minipig has become the gold standard for metabolic translational research however, the species and breed specific variation in hematologic parameters still needs be fully defined. Ossabaws are specifically known for their "thrifty" genotype that results in rapid intramuscular fat deposition and reduced muscle glycogen making it an efficient model for metabolic dysregulation (Kreutz et al., 2011; Matthan et al., 2018; Cluzel et al., 2022). When metabolic syndrome is induced Ossabaws will show high postprandial glucose, increased cholesterol and plasma triglyceride levels (Sturek et al., 2020). However, no studies have assessed the impact of dominance on Ossabaw blood parameters specifically. This is important as the exposure of swine to social stress has the potential to highlight physiological differences between dominant and submissive pigs. The most physically apparent difference being that dominant pigs are usually found to be larger than their submissive counterpart (Hicks, 1996; Chen et al., 2010; Lanthony et al., 2022). This leads to greater resource acquisition and a continued trend of larger daily weight gain. However, social interactions can be a source of chronic stress for both dominant and submissive pigs that can have an impact of health and functioning (Sutherland et al., 2006; Shen et al., 2020). Behavioral changes and aggression are often reflected in hematological measures such as an increased NL Ratio in dominant pigs and increased neutrophil percentage in subordinate pigs (McCrackin & Swindle, 2015).

The pigs outlined in this study were also exposed to multiple varieties of fermented sorghum tea (sumac, roasted sumac, white, and a mixture of all three). Fermented products carry many health benefits that can positively impact the blood parameters that become abnormal when metabolic syndrome is induced (Ham et al., 2019; Birhanu et al., 2021). This is largely due to the concentration of phenolic compounds, such as gallic acid, that are amplified when sorghum is put through the fermentation process. Commonly found in edible plants such as sorghum, gallic acid has anti-inflammatory, antioxidant, and metabolically protective properties which could also be impacting the pigs on the current study (Xu et al., 2021). The pigs were also exposed to a cognitive feeder test where they had to learn a pattern of paddles to press in order to gain a food reward. This test was run both individually and with their pen mate thus, testing their cognitive abilities when social status is applied.

The objectives of this project were to evaluate the hematological and chemistry panels of female and male Ossabaw pigs and determine the influence of social rank. The pigs on this experiment were exposed to a behavioral test and a preference test, therefore blood was evaluated both pre and post study. Blood was collected from the healthy pigs over a short period of time (~3 weeks). These were the same pigs utilized in the previous two studies. The changes seen in these blood parameters, while not showing great physiological impact, displays the preliminary effects of dominance and sorghum consumption on the porcine body. These effects will be better demonstrated in later work using both a lean and obese model. The overall combined effect of sorghum tea consumption and social status are considered as a summation of the preceding two studies.

Materials and Methods

Blood Collection

Blood was collected via the jugular vein at two different timepoints. Blood was first collected on May 26th, 2022, the day before testing began. The second samples were collected towards the end of the study on June 6th, 2022. Samples were allowed to clot for at least thirty minutes, then centrifuged at 2,000 xg for twenty minutes (Eppendorf, 5702 Low Speed Centrifuge, Enfield, CT) before being sent off for complete blood count (CBC) and chemistry testing (Antech Diagnostics, West Lafayette, IN).

Statistical Analysis

Statistical analyses were completed using SAS version 9.4 (SAS, Cary, North Carolina/USA, SAS Institute Inc.). A linear mixed model for repeated measures was utilized (Restricted-maximum likelihood; ANOVA). Included were the random variable of pigs and the fixed effect of status (dominant, submissive). The covariance for all analyses was compound symmetry. The outcome variables included body weight, consumption variables, complete blood count values, and chemistry values. Least-Squares means for all measures was assessed. All data was checked for normality using the Shapiro-Wilkes test in the UNIVARIATE procedure and was determined to be normally distributed. Pairwise comparisons were carried out using the two-sided test; the Tukey-Kramer method was used to adjust for multiple comparisons. Pigs were not exposed to exposed to the any fermented sorghum tea pre-study so consumption variables (Total Volume, mL, Gallic Acid, mg, Total Gallic Acid, µg/mL, and Total Iso Sugar Water) could not occur and so were analyzed utilizing the same model without the repeating time stipulation. Status, Session, and Status*Session interactions of P <0.05 were considered significant, and P > 0.05 < 0.10 was treated as a tendency when appropriate.

Results

General Results

Body weight possessed a significant Status*Time interaction as all pigs gained weight post-study and dominant pigs were heavier in comparison to submissive pigs (P=0.03; Table 4.1; Figure 4.1, A).

Consumption

There was no Status*Time interaction possible for this section of results, as pigs were not exposed to any of the liquid treatments pre-study. There was, however, a Status tendency for dominant pigs to have consumed a greater total volume of fluid than the submissive pigs (P=0.06; Table 4.1).

CBC (Complete Blood Counts)

There were no significant Status*Time interaction effects for any complete blood count values. Mean corpuscular volume (MCV) increased post-study (P =0.02; Table 4.1). There was a tendency to see an increase in mean corpuscular hemoglobin (MCH) post-study (P =0.08; Table 4.1). Mean corpuscular hemoglobin concentration (MCHC) decreased post-study (P =0.05; Table 4.1). There was a status tendency for Eosinophils, % to be higher in dominant pigs (P =0.08; Table 4.1). Finally, there was a session tendency for a decrease in Eosinophils, µL from pre to post study (P= 0.10; Table 4.1).

Chemistry

There were a small number of significant Status*Time interaction effects for blood chemistry values. Aspartate aminotransferase (AST) was higher in post-study dominant pigs for a significant Status*Time interaction (P =0.03; Table 4.1; Figure 4.1E). BUN/CREAT ratio was higher in pre-study submissive pigs (P =0.05; Table 4.1; Figure 4.1F). Chloride levels were highest in post-study submissive pigs (P =0.04; Table 4.1; Figure 4.1G). Cholesterol levels were lowest in dominant post-study pigs (P =0.04; Table 4.1; Figure 4.1B).

The pigs showed an increase in total protein, globulin, alanine transaminase (ALT), and amylase (P =0.03; Table 4.1). NaK ratio also had a tendency to be higher post-study (P =0.09; Table 4.1). In contrast there was a decrease post-study in gamma-glutamyl transferase (GGT), total bilirubin, blood urea nitrogen (BUN), and phosphorus ($P \leq 0.01$; Table 4.1). Albumin/globulin ratio also had a tendency to be lower post-study (P =0.08; Table 4.1). Glucose had a tendency for a Status*Time interaction with lower levels seen post-study and in submissive pigs (P =0.07; Table 4.1; Figure 4.1D). Precision PSL also had a tendency for a Status*Time effect with higher levels seen post-study and in dominant pigs (P =0.06; Table 4.1). Triglycerides had a tendency to be higher in dominant pigs (P =0.09; Table 4.1; Figure 4.1C). There was also a tendency for dominant pigs to have higher levels of creatine phosphokinase (CPK; P =0.08; Table 4.1).

Discussion

The aim of this study was to evaluate the possibility of hematological differences in dominant and submissive Ossabaw pigs that were exposed to fermented sorghum tea. This provides physiological context that will aid future work utilizing the feeder prototype and the further development of a fermented sorghum drink marketed for human consumption. The domestic pig has similar hematology to humans with the exception of lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC; Hanon et al., 1989; Sturek et al., 2007). However, on the current study both values were within the normal reference ranges for swine (Yeom et al., 2012; Zhang et al., 2022).

Social stress is known to impact feeding behavior and in turn health and performance (Sutherland et al., 2006; Rudine et al., 2007; Martínez-Miró et al., 2016). This is often seen through immune system dysregulation as dominant pigs exposed to normal stressors have shown higher white blood cell count indicative of inflammation (Sutherland et al., 2006). The dominant pigs on the current study had a tendency for higher eosinophil counts when compared to submissive pigs. This is could likely be connected through stress induced inflammation (Zheng et al., 2009). A stressed pig will also likely see an uptick in glucocorticoid release, which has hyperglycemic effects resulting in increased blood glucose levels (Fernandez et al., 1993; Tuchscherer et al., 1998). This presents the possibility that pens that had a higher level of agonistic interactions had a higher circulating blood glucose level on average and why dominant pigs had a higher blood glucose overall, as they displayed more aggressive behaviors. The stress that could have been created from a high conflict environment would contribute to higher glucose levels as well as evaluated levels along with other stress hormones have been identified in pigs after emotionally demanding situations (Otten et al., 2002; Choe & Kim, 2014). This could also be reflected in the dominant pig's tendency to have higher plasma creatinine phosphokinase (CPK) levels as this enzyme is secreted into the blood due to muscular injury and cell changes that occur under stress (Maxwell et al., 1976). While the impact of social status on dopamine signaling is not well understood, however there is evidence demonstrating lower plasma catecholamine concentrations in dominant mice (Fernandez et al., 1993). This higher value could also be attributed to the increase in exercise that was seen as the pigs were exposed to both the feeder and the maze as these muscles are activated at a greater rate due to testing as well as fighting in high conflict pens.

When comparing the more commercially utilized Yucatan to the Ossabaw it was found that the latter had increased triglycerides and higher glucose levels (Sturek et al., 2007). The pigs on the current study had triglyceride levels that tended to be higher in the dominant pigs than the submissive ones. This likely correlates with the larger size seen in the dominant pigs as well as a greater overall feed intake due to displacement. Results also found that Ossabaw pigs had increased creatinine as well as decreased urea nitrogen (Sturek et al., 2007).

Both dominant and submissive pigs saw a decrease in cholesterol from pre to post study. Dominant pigs had a higher level of cholesterol and in turn saw a larger decrease over time. As these were healthy pigs their cholesterol levels were consistently on the low side of normal for swine reference ranges (Bragagnolo et al., 2002; Sturek et al., 2007). The decrease in cholesterol levels could be partially attributed to the consumption of the fermented sorghum tea. The benefits of sorghum products amplified by fermentation have effective antioxidant properties that can reduce a hypercholesteremic state (Nisa et al., 2021). However, this relationship cannot be validated within the scope of this study, it does however allow the authors to conduct a more informed investigation in the future.

Ossabaw pigs subjected to a Western diet (high fat, high fructose), have developed metabolic syndrome without the presence of significant inflammation in their adipose tissue (Viera-Potter et al., 2015). The possibility of inducing metabolic syndrome independently of a high inflammation opens for the consideration of other contributing factors, such as social stress, to a high inflammatory state. Consumption of a beverage fortified with gallic acid has shown to reduce hypertension, dyslipidemia and liver dysfunction which are associated with metabolic syndrome (Xu et al., 2021). This could account for the decrease in AST that was seen in the dominant pigs as they did have a higher consumption of the tea overall. However, this does not explain the increase that was seen in the submissive pigs and may not be biologically relevant in this healthy population of pigs.

The induction of the Ossabaw pig into a metabolic syndrome will create an accurate model of the human prediabetic state (Lim et al., 2018; Matthan et al., 2018). The pigs on the current study were lean and pathology free. This creates a limitation in interpretation as effects would be more readily identified in an obese vs lean model. Due to the constraints of this testing, it is not possible to draw conclusions based up on these results. However, there were differences forming between dominant and submissive pigs that could have been largely driven by differences in feed intake and frequency of agonistic interactions. The true impact of a fermented sorghum beverage on hematology will have to be assessed in a subsequent study. This seems promising for a larger impact to be had on pigs that are experiencing metabolic syndrome on top of social stress.

Conclusions

The Ossabaw is a unique and valuable asset to biomedical and metabolic research. As many studies report on the state of an experimentally and metabolically altered population, these results can act as a reference for the baseline of a healthy Ossabaw minipig. This study also provides outlook on specific blood parameters that could be impacted by similar experimental conditions in order to provide additional context when interpreting results. No conclusions can be made in regard to the overall effect of fermented sorghum consumption, as there was not a control group that was exposed to the same testing procedures without also consuming the tea.

However, the preliminary work outlined above does help to lay a foundation for what can be expected when the pigs are exposed to similar testing procedures when under the influence of social stress and metabolic syndrome. The potential for physiological differences between dominant and submissive pigs in terms of body weight, cholesterol, triglycerides, and plasma glucose should be further explored in a lease vs. obese Ossabaw model.

Implications

The ability to identify pigs as dominant and submissive will allow for accurate prediction of their behavior and identification of metabolic risk factors both groups of pigs may be facing. This provides insight into not only the use of Ossabaws as a biomedical model but also the potential impact of social hierarchy on any experimental swine model. This will lead to an increase in welfare for experimentally utilized swine and the application of nuance to experimental results. Hopefully this will also create the opportunity for earlier intervention by pork producers with at risk herd members to prevent culling as social behavior is strongly linked to performance. Sorghum is also a common by-product of agricultural industry and has the potential to remedy the effects of metabolic syndrome on the pig. These results are limited by a small sample size run over a short period of time and will require further validation.

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Tables

Table 4.1. Hematological parameters of Ossabaw pigs pre and post study. Dominant and submissive status determined by dominance indices calculation.

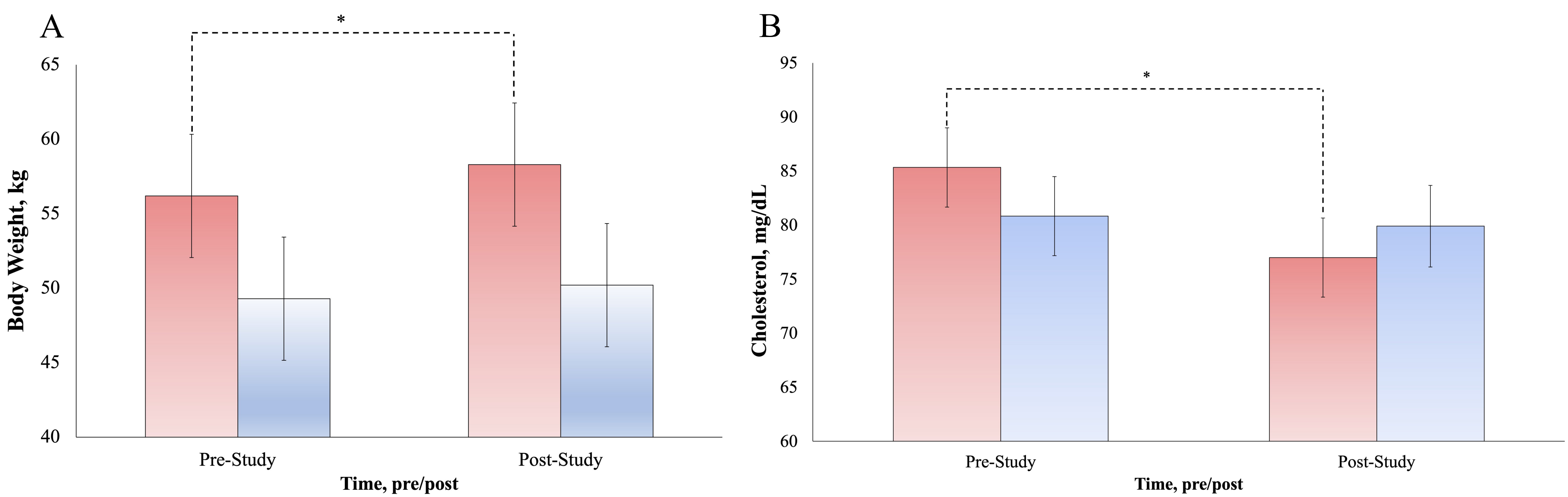
	Status			Time			*P*-Values		
Parameters	Dominant	Submissive	SEM[1]	Pre-Study	Post-Study	SEM[1]	Status	Session	Status x Session
Body Weight, kg	57.3	49.8	±4.13	52.75	54.26	±2.92	0.23	0.0001	0.03
Consumption									
Total Volume Tea, mL[2]	918.0	872.4	±16.18	-	-	-	0.06	-	-
Gallic Acid, mg[3]	78.4	76.3	±1.24	-	-	-	0.25	-	-
Total Gallic Acid, μg/mL[4]	287.8	296.6	±3.56	-	-	-	0.10	-	-
Total Iso Sugar Water, mL[5]	607.2	675.3	±75.98	-	-	-	0.52	-	-
Complete Blood Count									
WBC, μL[6]	9.9	10.38	±0.38	10.4	9.8	±0.34	0.37	0.18	0.39
RBC, μL[7]	9.0	8.7	±0.17	8.9	8.8	±0.14	0.30	0.46	0.70
HGB, g%[8]	18.1	18.2	±0.42	18.1	18.2	±0.32	0.92	0.55	0.42
HCT, %[9]	56.9	55.3	±1.18	55.4	56.8	±0.97	0.34	0.22	0.64
MCV, fL[10]	63.3	63.2	±0.81	62.1	64.4	±0.70	0.88	0.02	0.69
MCH, pg[11]	20.2	20.7	±0.31	20.3	20.7	±0.24	0.26	0.08	0.11
MCHC, g/dL[12]	32.1	32.8	±0.25	32.8	32.0	±0.25	0.07	0.05	0.08
Platelet Count, mL	157.4	162.0	±25.54	150.5	168.9	±20.21	0.90	0.30	0.98
Neutrophil, μL	3195.8	3173.3	±413.9	3212.	3156.2	±317.59	0.97	0.82	0.91
Neutrophil, %	32.4	30.5	±3.81	30.8	32.2	±2.82	0.73	0.42	0.72
NL Ratio[13]	0.6	0.6	±0.12	0.6	0.6	±0.09	0.76	0.84	0.70
Lymphocytes, μL	5709.8	6199.6	±489.9	6120.	5789.2	±366.78	0.50	0.21	0.18
Lymphocytes, %	57.58	60.27	±4.09	58.8	59.1	±2.98	0.65	0.81	0.92
Monocytes, μL	441.4	487.8	±43.68	509.1	420.1	±42.95	0.47	0.18	0.69
Monocytes, %	4.5	4.7	±0.44	4.9	4.3	±0.43	0.79	0.30	0.60
Eosinophils, μL	544.8	475.6	±45.25	583.0	437.3	±50.84	0.31	0.10	0.20
Eosinophils, %	5.5	4.5	±0.37	5.6	4.4	±0.43	0.08	0.12	0.25
Chemistry									
Total Protein, g/dL	7.9	7.9	±0.22	7.7	8.1	±0.17	0.96	0.03	0.73
Albumin, g/dL	4.2	4.4	±0.12	4.3	4.4	±0.09	0.33	0.51	0.98
Globulin, g/dL	3.6	3.5	±0.17	3.4	3.7	±0.13	0.55	0.01	0.63
A/G Ratio[14]	1.2	1.3	±0.07	1.3	1.2	±0.05	0.37	0.08	0.23
AST, IU/L[15]	41.2	37.3	±3.24	38.3	40.2	±2.73	0.42	0.53	0.03
ALT, IU/L[16]	45.5	41.5	±4.12	39.8	47.2	±3.17	0.51	0.01	0.48
Alk. Phosphatase	114.3	102.0	±16.87	110.4	105.9	±12.18	0.61	0.36	0.23

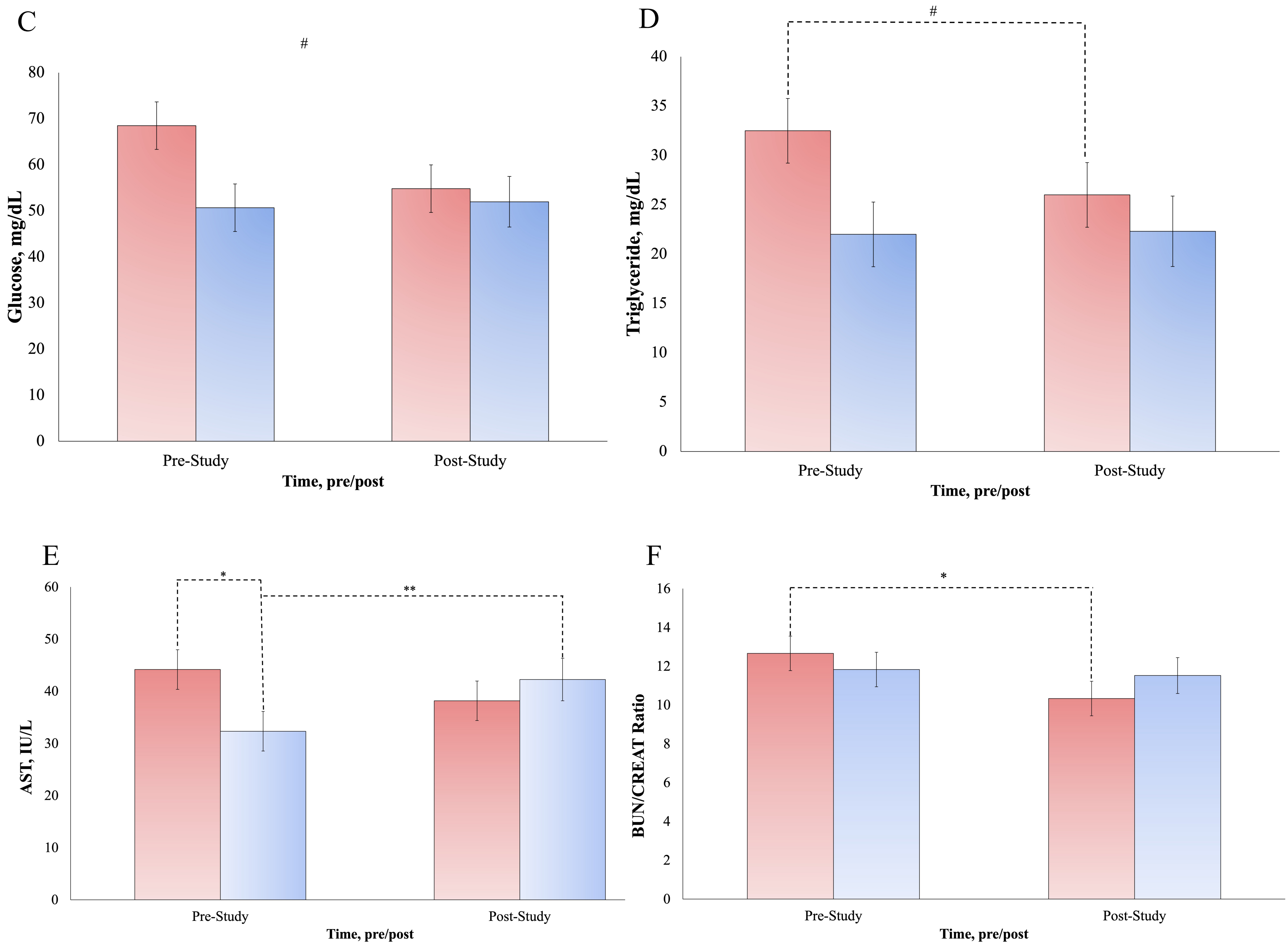
GGT, IU/L[17, 18]	56.3	55.9	±5.97	60.9	51.2	±4.47	0.96	0.01	0.70
BUN, mg/dL[19]	15.3	15.5	±1.05	16.5	14.3	±0.79	0.86	0.003	0.64
Creatinine, mg/dL	1.3	1.4	±0.07	1.4	1.3	±0.05	0.71	0.56	0.36
BUN/CREAT Ratio[20]	11.5	11.7	±0.84	12.3	10.9	±0.63	0.88	0.02	0.05
Phosphorus, mg/dL	10.3	10.5	±0.41	11.2	9.6	±0.38	0.75	0.01	0.40
Glucose, mg/dL	61.7	51.3	±4.59	59.6	53.4	±3.71	0.14	0.12	0.07
Calcium, mg/dL	11.0	11.0	±0.09	11.1	10.9	±0.10	0.71	0.24	0.60
Magnesium, mEq/L	2.7	2.6	±0.08	2.6	2.7	±0.08	0.60	0.45	0.83
Sodium, mEq/L	147.8	145.7	±1.10	145.8	147.6	±1.06	0.21	0.26	0.70
Potassium, mEq/L	6.6	8.0	±0.75	7.9	6.8	±0.63	0.22	0.12	0.78
NaK Ratio[21]	22.75	19.49	±1.45	19.8	22.5	±1.25	0.14	0.09	0.77
Chloride, mEq/L	100.0	101.2	±0.83	99.7	101.5	±0.70	0.33	0.04	0.04
Cholesterol, mg/dL	81.2	80.4	±3.54	83.1	78.4	±2.63	0.88	0.02	0.04
Triglyceride, mg/dL	29.3	22.2	±2.69	27.3	24.2	±2.42	0.09	0.32	0.27
Amylase, IU/L	1436.2	1053.1	±1229	1185.	1304.4	±8696.4	0.98	0.01	0.54
Precision PSL, IU/L[22]	10.4	10.5	1.20	10.4	10.5	±0.93	0.97	0.93	0.06
CPK, IU/L[23]	956.4	574.1	±138.5	891.7	638.8	±154.68	0.08	0.32	0.28

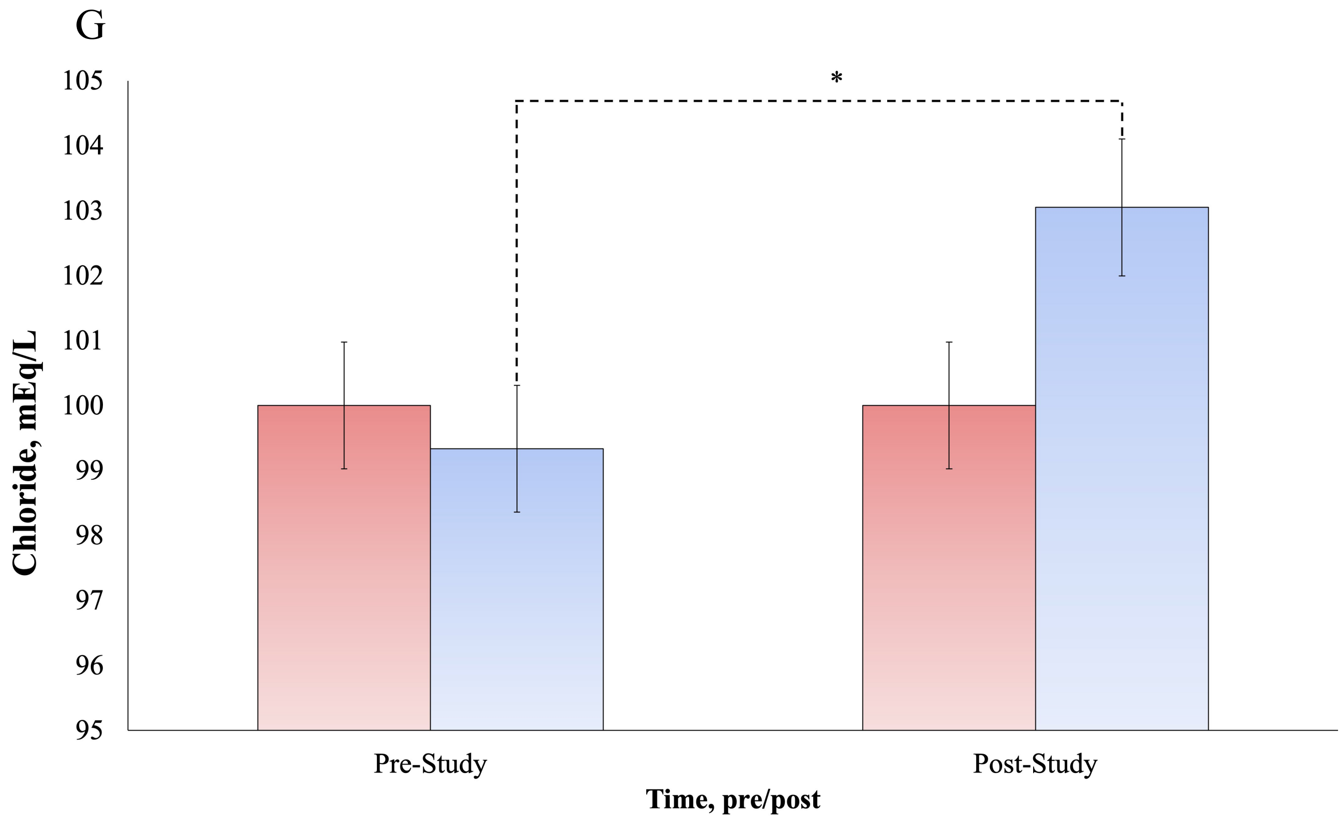
[1]Largest Standard Error Mean; [2]Total Volume of tea consumed; [3]Gallic acid concentration consumed in milligrams; [4]Total gallic acid concentration consumed in micrograms per milliliter; [5]Total consumption of isocaloric sugar water (+ control); [6]White blood cells; [7]Red blood cells; [8]Hemoglobin; [9]Hematocrit; [10]Mean corpuscular volume; [11]Mean corpuscular hemoglobin; [12]Mean corpuscular hemoglobin concentration; [13]Neutrophil/Lymphocyte Ratio; [14]Albumin/Globulin Ratio; [15]Aspartate Aminotransferase; [16]Alanine Transaminase; [17]Gamma-Glutamyl Transferase; [18]Total bilirubin stayed consistent for all pigs throughout the study with a value of 0.1 mg/dL. [19]Blood Urea Nitrogen; [20]Blood Urea Nitrogen/Creatinine Ratio; [21]Sodium/Potassium Ratio; [22]Pancrease Specific Lipase; [23]Creatine Phosphokinase.

Figures

Figure 4.1. Bar graphs denoting significant Status by Session interactions for the blood parameters of the pigs (n=11). (A) Body weight, kg for dominant and submissive pigs pre and post study. *Status*Session LS means differ (P <0.05; Tukey-Kramer adjustment). (B) Cholesterol, mg/dL for dominant and submissive pigs pre and post study. (C) Plasma Glucose, mg/dL for dominant and submissive pigs pre and post study. (D) Triglyceride, mg/dL for dominant and submissive pigs pre and post study. (E) AST, IU/L for dominant and submissive pigs pre and post study. (F) BUNCREAT Ratio for dominant and submissive pigs pre and post study. (G) Chloride, mEq/L for dominant and submissive pigs pre and post study. **LS Means for AST between pre-study submissive and post study submissive pigs (P =0.05). Status*Session for Glucose and Status for Triglyceride (P≥0.05≤0.1). Dominant pigs are represented by the red bars and submissive pigs are represented by the blue bars. Error bars represent SEM.




C
#
Glucose, mg/dL
0
10
20
30
40
50
60
70
80
Pre-Study
Post-Study
Time, pre/post
D
#
Triglyceride, mg/dL
0
5
10
15
20
25
30
35
40
Pre-Study
Post-Study
Time, pre/post
E
*
**
AST, IU/L
0
10
20
30
40
50
60
Pre-Study
Post-Study
Time, pre/post
F
*
BUN/CREAT Ratio
0
2
4
6
8
10
12
14
16
Pre-Study
Post-Study
Time, pre/post


G
*
105
104
103
102
101
100
99
98
97
96
95
Chloride, mEq/L
Pre-Study
Post-Study
Time, pre/post